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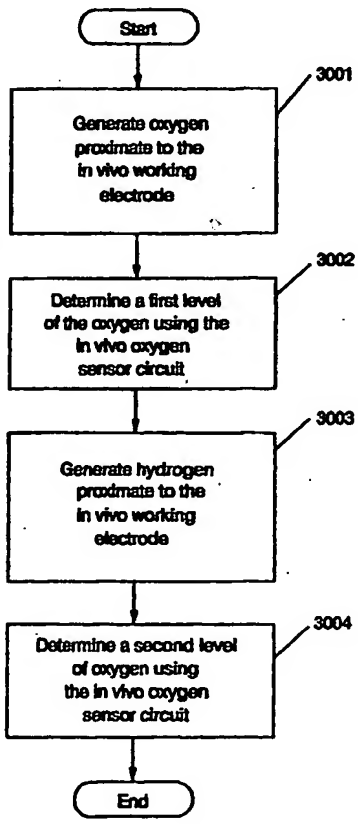

(57) Abstract: Calibration of in vivo oxygen and pH- sensor systems can be performed by generating a constituent element of an environment proximate to an in vivo sensor electrode via an in vivo generating electrode and determining a level of the constituent element in the tissue via the in vivo sensor electrode. Accordingly, accurate monitoring of tissue can be achieved while reducing the need to calibrate the in vivo sensor systems using invasive procedures. Related electrode assemblies are also discussed.

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METHODS OF CALIBRATING IN VIVO SENSOR SYSTEMS USING IN VIVO GENERATING ELECTRODES AND RELATED DEVICES

Related Applications

The present application claims priority from U.S. Patent Application No. 09/407,359 entitled "Methods and Systems and Associated Implantable Devices For Dynamic Monitoring of Physiological and Biological Properties of Tumors" filed on September 29, 1999.

Field of the Invention

The present invention relates to in vivo sensors, and more particularly, to tissue-implanted devices.

Background of the Invention

The availability of a system and device capable of monitoring changes within any cell population of interest would be an important addition to the cancer treatment armamentarium and one that will fill a need by making available more precise knowledge of the most sensitive time(s) for treating a tumor cell population. This vital information could aid in the delivery of highly specific individual treatment regime rather than the empirical and somewhat generalized treatment plans of today.

The *in vitro* study of malignant cell populations has established important general principles by which clinical treatment protocols are developed. These principles have established differences between malignant and normal cell populations and have been employed in the treatment of malignant disease. There have been many attempts to exploit these differences, both in pre-clinical and clinical studies, in order to attempt to obtain total tumor cell kill and improved cure rates. One of the major obstacles in achieving this goal has been the difficulty in minimizing normal tissue toxicity while increasing tumor cell kill (therapeutic index). Thus, presently, most treatment strategies employ an empirical approach in the treatment of malignant disease. That is, the timing of delivery and dose of cytotoxic agents are guided more by the response and toxicity to normal tissue than by the effects on the malignant cell population. A major deficiency of this empirical approach is the lack of an efficient method or technique to provide accurate information on the dynamic changes during treatment (which can be extended over a long period of time) that occur within a malignant cell population. Making this invaluable information available to attending physicians can allow clinicians to exploit the revealed

differences between malignant and normal cells, and hence improve the treatment procedures, to achieve better outcomes.

Much of the research in tumor biology has been involved in exploring the cellular, biochemical, and molecular difference between tumor and normal cells in order to improve the therapeutic index. Early cell kinetic studies revealed that cancer cells do not divide faster than normal cells, but rather a larger proportion of the cell population is dividing (Young et al., 1970). At that time, the failure to cure more tumors was attributed to a variation in growth characteristics. In the 1980's, it was proposed that these failures were due to development of resistance of tumor cells through mutations of an unstable genome (Goldie et al., 1984). Later studies suggested that the mechanism for tumor cell survival rests on expression of a gene that codes for a specific protein that expels or extrudes the cytotoxic agents from the cell (Chaudhary et al., 1992). More recently, it has been suggested that resistance is related to dysregulation of the cell cycle which alters the rates of cell growth (Lowe et al., 1994). Additional factors associated with failure to eliminate or effect improved cure rate include hypoxic cell populations, cell proliferation variants, cell differentiation agents, and cell cycle sensitive stages. The ability to monitor these changes during and following any treatment could offer a more precise knowledge of the most sensitive portions of any cell population and aid in the delivery of a more individualized and less empirical or generalized treatment program.

There have been a number of attempts to study certain of the dynamic changes occurring within a cell population, but these attempts generally lack the ability to monitor the changes on a real time basis. Indeed, these methods typically provide information at one point in time and most are designed to provide information on one particular function or parameter. In addition, most of the conventional methods can be expensive as well as time consuming. This can be problematic for patients undergoing extended treatment periods typical of radiation and or drug or chemotherapy, especially when it is desirable to follow dynamic changes both during an active treatment and subsequent to the active treatment throughout a treatment period.

The most reliable current monitoring technique is the biopsy. A biopsy can be taken at any time and can provide significant amount of information. However, it is impractical to biopsy each day and, even if one could, the time delay created in performing the various tests on the sample means that the information received by the physician is not an accurate representation of the patient's current condition. In addition to biopsy material, the radiological techniques of NMR and PET scanning can obtain, respectively, specific biological (cell cycle phase) and physiological (phosphorus) information, but both are sufficiently expensive that repetitive or daily

information is rarely available. The radioactive labeling of specific antibodies or ligands is another available technique, but this method has many of the same problems noted above with the other assays.

In addition, over time, tumors progress through periods wherein they are less robust and, thus, potentially more susceptible to treatment by radiation or drug therapy. Providing a monitoring system which can continuously or semi-continuously monitor and potentially identify such a susceptible condition could provide welcome increases in tumor destruction rates. Further, especially for regionally targeted tumor treatment therapies, it can be difficult to ascertain whether the desired dose was received at the tumor site, and if so received, it can be difficult to assess its efficacy in a relatively non-invasive manner. Thus, there is a need for a monitoring system which can quantify and/or assess the localized or regional presence of a target drug.

Although much of the particular tumor-specific and/or internal systemic information which may definitively identify the most vulnerable tumor stage and, thus, the preferred active treatment period, is still relatively unsettled (as is the ultimate definitive cure or treatment protocol), various researchers have proposed several potentially important physiological and/or biological parameters such as oxygenation, pH, and cell proliferation which may relate to tumor vulnerability or susceptibility, and thus impact certain treatment strategies.

For example, in the article "Oxygen tension measurements of tumors growing in mice," it is proposed that it may be helpful to assess hypoxia in tumors during treatment. Adam et al., Int. J. Radiation Oncology Biol. Phys., Vol. 45, 1998, pp. 171-180. In addition, tumor hypoxia has been proposed to have an impact on the effectiveness of radiation therapy. *See* Seminars in Radiation Oncology, Vol. 8, 1998, pp. 141-142. Similarly, the authors of "Development of targeting hyperthermia on prostatic carcinoma and the role of hyperthermia in clinical treatment" note that there is a need for a way to assess temperature at the site of the tumor during therapy. Ueda et al., Jpn. J. Hyperthermic Oncol., Vol. 15 (supplement), 1999, pp. 18-19. Moreover, Robinson et al. opines that it is important to know the tumor oxygenation level and blood flow. *See* Robinson et al., "MRI techniques for monitoring changes in tumor oxygenation in blood flow," Seminars in Radiation Oncology, Vol. 8, 1998, pp. 197-207. Unfortunately, tumor oxygenation can vary and there is evidence to suggest that tumor oxygenation is in a continuous state of flux. *See* Dewhirst, "Concepts of oxygen transport at the microcirculatory level," Seminars in Radiation Oncology, Vol. 8, 1998, pp. 143-150. This flux makes a dynamic monitoring method important for identifying when the tumor oxygenation level is such that a more active treatment strategy may be desired. In addition, tumor pH has been suggested as an exploitable parameter for drug design for tumor treatments. *See* Leo E. Gerweck, "Tumor pH:

Implications for Treatment and Novel Drug Design", 8 Seminars in Radiation Oncology No. 5, pp. 176-182 (July 1998).

In the past, various biotelemetry devices and implantable sensors have been proposed to monitor cardiac conditions or physiological parameters associated with glucose or temperature. For example, U.S Patent No. 5,791,344 to Schulman et al. entitled "Patient Monitoring System," proposes a system to monitor the concentration of a substance in a subject's blood wherein one enzymatic sensor is inserted into a patient to monitor glucose and then deliver insulin in response thereto. Similarly, PCT US98 05965 to Schulman et al, entitled "System of Implantable Devices for Monitoring or Affecting Body Parameters," proposes using microsensors and/or microstimulators to sense glucose level, O_2 content, temperature, etc. There are also a number of implantable medical devices and systems which monitor physiological data associated with the heart via telemetry. One example of this type of device is described in U.S. Patent No. 5,720,771 to Snell entitled, "Method and Apparatus for Monitoring Physiological Data From an Implantable Medical Device." The contents of these applications are hereby incorporated by reference as if recited in full herein.

In addition, unlike conventional implanted sensors, tumor monitoring systems and/or sensors used to monitor tumors can be exposed to a relatively harsh environment during a treatment protocol or strategy which can extend over a period of weeks, or even months (such as applied heat, chemicals and/or radiation). Further, such a harsh environment, coupled with an extended treatment period, can affect the function of the device and thus, potentially corrupt the measurement data it generates.

Moreover, chronically implanted sensors can be susceptible to drift or loss of sensitivity over time. For example, the accuracy of some in vivo sensors may degrade due to bio-fouling and/or degeneration of the sensors or drift in the electronics of the sensor. Some conventional sensors are, therefore, calibrated using external devices. For example, some conventional sensors are calibrated using arterialisation of subcutaneous oxygen tension as discussed, for example in "Arterialisation of Transcutaneous Oxygen and Carbon Dioxide," Archives of Disease in Childhood, Vol. 63, 1988, pp. 1395 - 1396, by Broadhurst et al. Other sensors can be calibrated using a calibration reagent that is injected near the sensor as discussed, for example, in "In Vivo Evaluation of Monopolar Intravascular pO_2 Electrodes," Pediatric Research, Vol. 13, 1979, pp. 821-826, by Beran et al. The reagent may have a known composition which may allow the data generated by the sensor to be compared to an idealized value associated with the reagent.

Other conventional sensors can be calibrated by periodically analyzing tissue taken from near the sensor as discussed, for example, in "Continuos Comparison of In Vitro and In Vivo Calibrated Transcutaneous Oxygen Tension With Arterial Oxygen

Tension Infants," Birth Defects: Original Article Series, Vol. XV, No. 4, 1979, pp295-304, by Pollitzer et al. The data derived from the analysis can be compared to the data generated by the sensor. Unfortunately, these conventional calibration methods may be inaccurate or may involve frequent invasive procedures to perform the calibration.

In view of the foregoing, there remains a need for tumor monitoring systems and devices which can, *inter alia*, monitor the physiological and/or biological condition of a tumor during a treatment cycle to identify enhanced or favorable treatment windows to potentially increase *in vivo* treatment efficacy associated with such treatment.

There also continues to be a need for improved methods of calibrating in vivo sensor circuits and related in vivo devices.

Summary of the Invention

The present invention can provide improved methods of calibrating in vivo sensor circuits by generating a constituent element of an environment proximate to an in vivo sensor electrode via an in vivo generating electrode and determining a level of the constituent element in the tissue via the in vivo sensor electrode. Accordingly, long-term monitoring of tissue can be achieved while reducing the need to calibrate the in vivo sensor using invasive procedures.

In one embodiment, an in vivo sensor system can be calibrated by generating a first constituent element of an environment proximate to an in vivo sensor electrode at an in vivo generating electrode. A first level of the first constituent element is determined via the in vivo sensor electrode. A second constituent element of the environment is generated proximate to the in vivo sensor electrode at the in vivo generating electrode. Finally a second level of the second constituent element is determined using an in vivo sensor electrode.

In another embodiment, calibration is achieved by determining a relationship between a current in the environment and a third level of the first constituent element using the first and second levels of the constituent elements.

In another embodiment, the first constituent element is oxygen and the second constituent element is hydrogen.

In yet another embodiment, an in vivo oxygen sensor system can be calibrated by generating oxygen proximate to an in vivo sensor electrode at an in vivo generating electrode. A first level of the oxygen is determined via the in vivo sensor electrode. Next, hydrogen is generated proximate to the in vivo sensor electrode at the in vivo generating electrode and a second level of the hydrogen is determined using the in vivo sensor electrode.

In still another embodiment, an in vivo pH sensor system can be calibrated by generating a changing H^+/OH^- ion level proximate to an in vivo sensor electrode. A rate of change of a voltage is determined at the in vivo sensor electrode generated in response to the changing H^+/OH^- ion level. Finally, a rate of change of the voltage associated with the changing H^+/OH^- ion level is determined. In a further embodiment, the rate of change of the voltage is associated with a voltage level that is associated with a pH level.

In still another embodiment, an increase in OH^- ions and a decrease in H^+ ions is generated proximate to the in vivo sensor electrode and a decrease in OH^- ions and an increase in H^+ ions is generated proximate to the in vivo sensor electrode.

Brief Description of the Drawings

Figure 1A is a schematic illustration of a tumor monitoring system according to the present invention. The illustration portrays a real-time monitoring capability.

Figure 1B is a schematic illustration of an alternate tumor monitoring system according to the present invention. This figure illustrates an ongoing dynamic remote monitoring capability.

Figure 2A is a schematic diagram of a tumor monitoring system configured to relay real time tumor information during an active treatment session (shown as an electric field treatment therapy) according to one embodiment of the present invention.

Figure 2B is a block diagram illustrating a tumor monitoring system configured to relay information (real-time) during a hyperthermia and radiation treatment session.

Figure 3 is a block diagram of a method of monitoring a tumor undergoing treatment according to the present invention.

Figure 4 is a flow chart of a method to identify favorable and unfavorable treatment times according to the periodic (dynamic) monitoring of a plurality of tumor physiological parameters according to the present invention.

Figure 5 is a top view of an implantable biocompatible sensor according to the present invention.

Figure 6A is a top view of an alternative implantable biocompatible sensor according to the present invention.

Figure 6B is a side view of the sensor shown in **Figure 6A**.

Figure 7 is a side section view of an injectable microsensor according to the present invention.

Figure 8A is a section view of the sensor shown in **Figure 7** taken along line **8A-8A**.

Figure 8B is a front perspective view of an alternative embodiment of an injectable microsensor similar to the embodiment shown in **Figure 7**.

Figure 9 is a schematic illustration of an implant sensor according to another embodiment of the present invention.

Figure 10A is a greatly enlarged cutaway front view of a mock implant of a pH sensor with a pH (ionophore) membrane according to the present invention.

Figure 10B is a side view of an alternate embodiment of a pH sensor (with iridium oxide).

Figure 11 is a schematic illustration of an experimental setup used to evaluate an implant tumor sensor according to the present invention.

Figure 12 is a block diagram of a circuit for an implantable sensor according to the present invention.

Figure 13 is a graph of the operation of an exemplary transmitter according to the present invention.

Figures 14A-C are graphs illustrating transmitter operational parameters according to one embodiment of the present invention. **Figure 14A** illustrates capacitor voltage over time, **Figure 14B** illustrates control voltage over time, and **Figure 14C** illustrates an output voltage waveform.

Figure 15 illustrates an IC block diagram according to one embodiment of the present invention.

Figure 16 is a pictorial representation of an IC layout corresponding to **Figure 15**.

Figures 17A and **17B** are graphs of the results of IC prototype temperature experiments. **Figure 17A** illustrates temperature versus pulse width of data corresponding to a thermistor (with the chip inside a water bath of varying temperature). **Figure 17B** illustrates temperature versus pulse width of data corresponding to a fixed resistor (also with the chip inside a water bath of varying temperature).

Figures 18A and **18B** are graphs of the results of IC prototype radiation experiments. **Figure 18A** illustrates pulse width versus radiation of data corresponding to the thermistor with the chip inside the water bath and exposed to radiation from about 0-8000 cGray (a patient is typically treated with radiation in the range of about 3000-6000 cGray). **Figure 18B** illustrates the data corresponding to the fixed resistor data with the chip inside the water bath and exposed to radiation from about 0-8000 cGray.

Figure 19A is a schematic illustration of a subject with monitoring system with two separate and spaced apart implant sensors positioned on two different tumors according to one embodiment of the present invention. The monitoring system

receiver can refocus to monitor both locations and transmit the data to a remote location.

Figure 19B illustrates an implant sensor with four sensor elements in position (*in situ in vivo*) according to one embodiment of the present invention. As shown, two of the sensor elements are positioned at different surface locations on the tumor, while one of the sensor elements is positioned to penetrate a depth into the tumor. Still another of the sensor elements is positioned proximate to normal tissue that is proximate to the malignant tissue or tumor.

Figure 20 is a schematic illustration of a self-calibrating *in situ, in vivo* microsensor.

Figure 21 is a photograph of a self-calibrating oxygen sensor.

Figure 22 is a section view of a self-calibrating combination pH and O_2 sensor.

Figures 23A-23C are side views of the sensor of **Figure 22** illustrating a fabrication sequence.

Figure 24 is a block diagram of an embodiment of an in vivo oxygen sensor system according to the present invention.

Figures 25A and **25B** are schematic diagrams of in vivo oxygen sensor circuits coupled to electrodes of in vivo oxygen sensor assemblies according to the present invention.

Figure 26 is enlarged illustration of a first embodiment of an oxygen electrode assembly according to the present invention.

Figure 27 is an enlarged illustration of a second embodiment of an oxygen electrode assembly according to the present invention.

Figure 28 is enlarged illustration of a third embodiment of an oxygen electrode assembly according to the present invention.

Figure 29 is an enlarged cross-sectional view of an embodiment of a portion of an electrode assembly according to the present invention.

Figure 30 is a flow chart that illustrates operations of embodiments of oxygen sensor assemblies according to the present invention.

Figure 31 is a block diagram of embodiment of an in vivo pH sensor system according to the present invention.

Figure 32 is an illustration of a pH titration curve according to the present invention.

Figure 33 is enlarged illustration of a first embodiment of a pH electrode assembly according to the present invention.

Figure 34 is enlarged illustration of a second embodiment of a pH electrode assembly according to the present invention.

Figure 35 is a flow chart that illustrates operations of embodiments of pH sensor assemblies according to the present invention.

Detailed Description of the Invention

The present invention now will be described more fully hereinafter with reference to the accompanying drawings, in which preferred embodiments of the invention are shown. This invention may, however, be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art. Like numbers refer to like elements throughout. In the figures, certain layers, regions, or components may be exaggerated or enlarged for clarity.

Generally stated, the systems, devices, and methods of the present invention are aimed at monitoring the changes in physiology and kinetics of living systems. More specifically, the present invention's goal is to monitor at sufficient intervals (preferably semi-continuously, and more preferably substantially continuously) the changes in oxygen, pH, and cell proliferation of any organ or tumor system under "normal" physiological conditions, *in-situ*, as well as prior to, during and following any perturbation (such as radiation, chemical or cytotoxic stimuli and hyperthermia) of such systems. As such, the monitoring systems and methods of the present invention can be useful in many applications, such as, for example, pulmonary, gastrointestinal, neuroscience and pre-clinical research. Nonetheless, the present invention has a particular importance and suitability to tumor systems. As such, the following description of preferred embodiments will primarily discuss the utilization of the present invention for cancer applications.

As noted above in the Background of the Invention, most conventional cancer treatment strategies employ an empirical approach. That is, the timing and delivery of cytotoxic agents are guided more by the response and toxicity to normal tissue than by the effects on the malignant cell population. Thus, a major deficiency of this empirical approach is the lack of an efficient method or technique to provide accurate information on the dynamic changes during treatment that occurs within a malignant cell population. Making this invaluable information available to attending physicians will allow them to exploit the revealed differences between malignant and normal cells, and hence improve the treatment procedures to achieve better outcomes. Conventionally, the normal tissue surrounding the tumor governs the dose of radiation and the scheduling and doses of chemotherapy is most dependent on the tolerance of the patient's bone marrow. The primary reason for the lack of individualization of treatment is that there is presently no commercially viable means by which the basic

information on kinetics and physiology of the tumor can be obtained during and following treatment. A biopsy of the tumor will yield information at one point in time and therefore is valid for only that point in time. This static "snapshot" information may not be valid for predicting the cell kinetics, especially cell kinetics following perturbation by any cytotoxic agent.

There have been a number of attempts to study the dynamic changes occurring within a cell population. However, these lack the ability to monitor the changes on a real time basis. Instead, the conventional methods provide information at one point in time, most are designed to provide information on one function, and most are expensive and time consuming, especially when one considers that it is important to monitor parameters before, during, and following treatment.

The major goal of cancer therapy is to eliminate all tumor cells. Knowledge of the specific change occurring within the tumor at substantially any time can be desirable in order to achieve maximum tumor cell kill and minimum normal tissue damage. Cytotoxic agents are most effective at specific times and conditions of tumor growth. If the most vulnerable time of the tumor cells can be determined, *i.e.*, the time of maximum oxygenation or identification of an increase in cell proliferation associated with phases of the cell cycle, then this information can be used to direct the time of delivery and the choice of the cytotoxic agents.

Preclinical and clinical medicine are in need of information on the dynamic changes which occur in malignant tissue prior to, during, and following cytotoxic (active) therapy sessions in order to define more clearly the circumstances for increasing tumor response. Access to such information can allow for more precise timing of the delivery of cytotoxic agents as well as identifying the most appropriate agent(s), *e.g.*, radiation or chemotherapy therapy. Conventional radiological investigations are limited by their ability to observe dynamic changes, although NMR and PET scan can identify some functional changes. The currently available anticancer agents, although effective in a limited number of tumors, are relatively ineffective in the majority of cancers. The instant invention recognizes that the reasons for this lack of improvement in outcome are typically multifactorial and related in part to an inability to measure, *in situ*, the time profiles of the most sensitive parameters. These tumor parameters include one or more of, but are not limited to, the degree of oxygenation, pH, cell cycle phases, cell proliferation, and the molecular and cellular determinants of sensitivity or resistance to cytotoxic agents. The present invention recognizes that the availability of such information and the ability to act upon such information can provide the means of overcoming a major barrier to improvements in outcome in cancer therapy. Further, it is believed that this newly provided information can create a shift in the therapeutic paradigm from empirical to

individual based therapy which can rely (at least in part) on the molecular and cellular properties of the individual patient's tumor.

Advantageously, the present invention now can provide information on the changes occurring during and after therapy which can be utilized to direct therapy and/or to monitor the effects of the therapy. This individualization of therapy can not only improve outcome but also decrease toxicity and morbidity of the treatment. That is, the information obtained on each patient's tumor can radically change the scheduling of therapy and result in an improved outcome. For example, patients can now be monitored from home, through telephone lines or some other remote interface, to determine a favorable or most appropriate time for treatment

Thus, as noted above, the present invention is primarily directed to the *in vivo* evaluation and monitoring of tumors prior to, during, and subsequent to an active treatment, and preferably over an entire treatment regime or period. That is, the present invention is particularly suitable for monitoring the behavior of cancerous tumors such as sarcomas and carcinomas over a particular non-remission treatment period. As such, the internal *in situ* sensors of the present invention are preferably configured to be biocompatible and provide a service life suitable for episodic treatment evaluation of at least about 4-6 weeks, and more preferably at least about 6-10 weeks, and still more preferably at least about 10-12 weeks, whether exposed to radiation, chemotherapy, heat or ionic electric fields (such as the treatment provided by a Thermotron®) directed to the tumor. The sensors and preferred tumor monitoring parameters will be discussed further below.

Turning now to **Figure 1A**, a real-time tumor monitoring system **10** is illustrated. As shown, the tumor monitoring system **10** includes an *in situ* sensor unit **50** positioned in a subject **20** proximate to a tumor **25**. Preferably, as is also shown, the sensor unit **50** includes a plurality of sensor elements **51** positioned at different locations on and/or into the tumor **25**. It is preferred that the sensor elements **51** monitor more than one physiological parameter or a selected physiological parameter associated with the tumor at more than one position in, on, or about the tumor as will be discussed further below. The sensor unit **50** is configured with a telemetry link **60** to wirelessly communicate with an externally located receiver **75**. The receiver **75** includes a computer interface **76** and is operably associated with a physician interface module **80** such as a display monitor associated with a central processing unit, computer, or other computer means to allow physician access to the monitored data. As shown, the physician interface **80** is a laptop or other mobile/portable computer means to allow a physician instant access to the substantially real-time monitored tumor parameters.

Figures 2A and **2B** illustrate exemplary applications of real-time evaluations according to the present invention. **Figure 2A** illustrates using the monitored parameter(s) of the tumor during a hyperthermia therapy session (such as via Thermotron® device) to control the length, power, field strength, or polarity of the treatment. This control can be provided because the real-time monitored data associated with at least one tumor parameter can provide feedback on the actual treatment penetration depth (via temperature or other parameter) at the tumor itself. Alternatively, the information regarding the condition or behavior of the tumor may suggest another treatment would be more beneficial, or even that further treatment would not be beneficial (at that time). Indeed, it is preferred that prior to initiation of any active treatment, the tumor data is monitored to assess whether conditions are favorable or indeed, unfavorable, for the treatment strategy proposed. That is, if a drug therapy is recommended for tumors exhibiting a pH above a certain value, and the data suggests that the tumor pH is below this value, a physician may choose to postpone that particular therapy for a more favorable time. Of course, other parameters, such as an elevated oxygenation level and a phase of increased cell proliferation, may suggest that other therapy would be more advantageous or that the drug therapy should nonetheless proceed. Additional discussion regarding tumor parameters and the relationship to treatment is provided below.

Figure 2B illustrates the use of the real-time tumor data in a control feedback loop to control one or more of the power, dose, or duration of a hyperthermia and radiation treatment session. As shown the monitored transmitted data is sent to the receiver **75** which then inputs the data into a computer which has a controller directing the actuator **92** and treatment source **91** (which directs the treatment into the patient). The patient **20** is noted as the controlled "plant" in this figure.

Figure 1B illustrates an alternate embodiment of a tumor monitoring system **10'**. In this embodiment, the tumor monitoring system **10'** includes a home receiver unit **75'** and a remote interface **78** which communicates with the physician interface **80** (the physician interface shown in this embodiment is a central processing unit). The patient **20** (the dotted line represents the patient being in the house proximate to the receiver **75'**) even when at home can continue to monitor and transmit data to a remote site. The remote interface **78** can provide the communications link between the monitored local data and a remote clinical oversight station. As such, the remote interface **78** can be provided by any number of interface or data load means including a computer modem, a wireless communication system, an internet connection, or telephone connection. In this embodiment, upon identification of the existence or onset of a favorable condition for treatment, the central processing site can automatically schedule an evaluation appointment or even schedule a treatment

session on therapeutic equipment to take advantage of an opportune or favorable treatment window(s).

Figure 3 illustrates a preferred tumor monitoring and treatment evaluation method according to the present invention. At least one (and preferably a plurality of) physiological parameter associated with a tumor in a subject undergoing treatment is monitored (**Block 100**). Data associated with the at least one physiological parameter is transmitted from an *in situ* positioned sensor unit **50** to a receiver **75** located external to a subject (**Block 110**). The data transmission can be remotely transmitted from a non-clinical site (such as at a patient's home) to a clinical site via modem, telephone, wireless communication systems, and the like (**Block 115**). The transmitted data is then analyzed to determine a condition of the tumor (**Block 120**). The monitoring, transmitting, and analyzing steps are repeated at a plurality of sequential points in time (**Block 125**). That is, as opposed to a "static" single point in time data point, the instant invention allows dynamic monitoring (a plurality of sequential points in time). The dynamic tracking to variation in the tumor can yield valuable therapeutic and diagnostic information. The data is transmitted on a periodic basis (such as every 4-24 hours) over a particular treatment period. The data is transmitted in an at least an intermittent manner (although the data may be transmitted in less or more frequent data transmissions) during an entire treatment cycle, typically from about 1-3 months. More preferably, the data is substantially continuously or semi-continuously monitored (every 1-60 minutes, and more preferably every 1-30 minutes) and, at least locally, transmitted. This ongoing (intermittent, semi-continuous, or substantially continuous) monitoring allows the dynamic tracking or monitoring of the physiological parameter(s).

Of course, the continuous or semi-continuous monitoring/transmitting can be performed locally for electronic storage within memory associated with the receiver/computer interface **75'** and then subsequently transmitted (to a central monitoring site on a less frequent basis, such as hourly, daily, and the like). It may be beneficial to preset a data transmittal/acquisition time via a timer in communication with the receiver **75'** corresponding to a physician's input (*e.g.*, more frequent monitoring closer in time to the introduction of cytoxic agents or pertubation, such as every 1-5 minutes, with less frequent monitoring subsequent thereto, such as every 10-15 minutes, or hourly). Alternatively, the data monitoring/transmitting or acquisition time may be self-adjusting and relatively set such as by comparing and reviewing the transmitted data periodically to determine rates of change upon which to institute a more frequent assessment, then transmit less frequently during times of less change in the values. In any event, for stationary receiver units **75**, **75'**, the patient needs to be in proximate position with the receiver **75'** to facilitate proper data transmittal. In

order to facilitate the proper position of the patient for a subsequent transmittal to the receiver **75'**, the receiver **75'** is preferably configured to generate an alert or alarm when a desired monitoring transmittal time is approaching. This can remind a subject to approach the receiver for proper transmission therebetween. Of course, the receiver **75'** can be programmed to audibly state the next transmitting time based on the values of the most recently transmitted data while the more current transmittal is still underway (or on the change between a series of more recent transmittals).

In an alternative embodiment to the home-based tumor monitoring system **10'** shown in **Figure 1B**, the receiver **75'** can be configured to be portable and sufficiently light weight to allow a user to wear it (attached to clothing or other supporting belts or suspenders or the like) such that it is in a desired proximity to the imbedded sensor unit(s) **50** to more easily provide semi-continuous or substantially continuous dynamic data tracking. Preferably, the portable receiver unit (not shown) is self-powered with a trickle charger (to plug into a vehicle accessory power source or a wall outlet in the home) to allow a user to recharge the unit when not mobile. It is also preferred that the portable unit be configured with sufficient memory to be able to store a block of data over a period of time before uploading to the remote interface, or directly to a computer interface at a clinical site.

In any event, referring again to **Figure 3**, a tumor treatment strategy can be evaluated based on the dynamic information provided by the monitored parameter(s) **(Block 130)**. This evaluation can result in a verification of the efficacy of a treatment **(Block 132)** such as, for example, to determine whether the tumor is responding or resistant to the treatment. Further, the evaluation can verify that a given active dose was received at the tumor and in what amount. One example is to quantify the amount of radiation seen or received at the tumor (this can be helpful if the tumor is blocked by dense tissue or is irregularly configured or positioned in the body in hard to reach regions). This verification may also be particularly suitable for use with newer targeted drugs which are designed to target the specific treatment zone in the body. This verification can thus affirm that the drug is delivered to the region intended.

In addition, the evaluation can be advantageously used to identify either, or both, of the presence of a favorable or unfavorable treatment time **(Block 134)**. For example, if conditions indicate the tumor is not receptive to the planned treatment, a change in the planned therapy can be promptly instituted, or, in the reverse, the resistance can result in a rescheduling of a planned therapy to a more favorable time, thereby minimizing exposing the subject to unnecessary therapy sessions at unfavorable times. In addition, the therapeutic evaluation can be based on either or both of relative or absolute parameter values (or indeed a clustering of irregular,

positive, or negative parameter values) to determine if the treatment is progressing according to a predictive model. The predictive model can be based on the deviation of the tumor's response to the delivered therapy at a particular point in time as measured against a population norm or even against a historical perspective of the patient's own responses to previously delivered therapies. This can allow a physician to choose (or modify) the therapy for a subject based on the responsiveness of the tumor itself. Thus, the information can result in modification of the planned treatment regime (**Block 136**). For example, for discussion purposes, assume that at Day 3 from a chemotherapy type and dose, the tumor oxygenation is low, and the normal cell's susceptibility to toxic agents is high. In contrast, assume that at Day 3, the tumor oxygenation is high, and the normal cell's susceptibility to toxic agents is low. In the latter, this behavior may be according to a predicted outcome or an unpredicted outcome; if unpredicted, one might proceed to schedule take advantage of the favorable conditions for treatment and schedule an additional therapy session promptly (*i.e.*, a favorable active treatment time). If predicted, then the planned therapy can proceed as scheduled.

Determining Tumor Physiological Parameters

It is generally well accepted that tumor oxygenation and blood flow are important to the efficacy of most types of cancer therapy. Hypoxia (low oxygen) and thus radiation resistance occurs in poorly perfused regions of tumors (Gray et al., 1953). In addition, anticancer drugs of all kinds gain access to tumor cells through blood vessels, and poorly perfused regions also hinder drug delivery (Jain et al., 1988). For these reasons, there has been great interest in developing methods for modifying and monitoring tumor blood flow and oxygenation, primarily to find ways to increase radiation sensitivity. However, a knowledge of tumor oxygen levels can lead to alternative approaches, *e.g.*, hyperthermia effects which are enhanced in hypoxia (Stratford et al., 1994). More recent information on the influence of hypoxia in the regulation of genes and cytokines has continued to stimulate interest in this area (Sutherland et al. 1994)). Further, it is likely that these effects are involved in influencing patterns of metastases (Young et al., 1997), angiogenesis (Schweiki et al., 1992) and drug resistance (Sakata, 1991).

Currently there is no commercially feasible clinically applicable noninvasive method of assessing tumor hypoxia (McCoy, 1996). Magnetic resonance imaging and positron emission (Robinson, 1998) have been discussed as possible means to monitor changes in tumor perfusion and blood oxygenation. However, these methods are cumbersome to monitor the daily and dynamic changes, which occur during the perturbation of a tumor. The ability to monitor tumor oxygenation and changes

within the tumor during various challenges is important to improve cancer therapy. The information obtained can direct the type of and timing of appropriate therapy, in order to increase the cytotoxic effect.

A substantial body of evidence has accumulated over the past 50 years indicating that electrode-evaluated human tumor pH is, on average, lower than the pH of normal tissue. However, strategies to explore this difference have been hampered for two reasons; first, overlap of electrode-measured tumor and normal tissue pH, especially when data is pooled. Second, more recent demonstration using 31P magnetic resonance spectroscopy (MRS) indicates that tissue pH can be divided into two compartments: intracellular and extracellular--(a) pH determined by electrodes primarily measure interstitial or extracellular pH and (b) pH determined by MRS primarily reflect intracellular pH ("pH_i"). Moreover, the pH_i of normal and tumor tissue is similar whereas the extracellular pH may vary significantly between normal tissue and tumor and tumor of the same origin but in different patients. For example, the range of pH in breast tumors has been demonstrated to be from 6.85-7.5 and in the subcutaneous tissue of normal volunteers it was from about 7.3-7.9.

The electrode-measured pH of tumors is on average 0.4 units lower than normal subcutaneous or muscle tissue. Although overlap between normal and tumor tissue may exist, they may be explained by technical and patient-related factors. However, the present invention recognizes that measuring pH in both normal and tumor tissue at the same time and on a continuous basis can eliminate this variation. The ability to accomplish this can enable the physician to exploit the differences. Since the acidity increases with increasing distance from the supplying vessel and pH_i is similar in each tissue, the intra to extra cellular pH gradient may be expected to increase in those cells most distal from blood vessels. The overall effect would be to enhance drug uptake and killing of cells that are normally exposed to the lowest drug concentration and especially relevant to radiation therapy in which low oxygen concentration -- and therefore radiation resistance -- increases with increased distance.

Accordingly, in one embodiment of the present invention, the sensor unit **50** (whether self-powered and implantable or injectable with an inductive powered version as will be discussed further below) can be inserted into the tumor(s) and secured therein or thereto in order to gather information, preferably for a number of weeks as discussed above. As shown in **Figure 19B**, the sensor elements **51** are configured such that they are placed at different levels and in different locations in the tumor. It is also preferred, as is also shown in **Figure 19B**, that at least one sensor element be configured to monitor the treatment toxic affect or normal cells and/or the pH level of the normal cell tissue proximate the tumor.

It has been shown that a difference in oxygen levels exist between tumor feeding arterioles (about 32mm Hg) as opposed to the about a 50mm Hg level in healing or normal tissues. And as noted above, low oxygen levels leads to treatment resistance in a tumor cell. If it is determined, with the aid of the device, that the majority of the tumor is hypoxic (*i.e.*, less than 50mm Hg, and preferably less than about 40mm Hg, and more preferably about 32mm Hg or less), then it should not be treated until the oxygenation of the tumor is improved. This can occur in several ways. The tumor can be heated (hyperthermia) which works best in hypoxic conditions and which may eliminate enough cells to make the remaining population less hypoxic, or the tumor can be exposed to specific drugs to improve the oxygen concentration. The important point is that the tumor is not treated until more cells are oxygenated and, therefore, more sensitive or vulnerable to the conventional active treatments of radiation or chemotherapy. Similarly, the sensitivity and, therefore, cell kill of malignant cells can be affected by pH and cell proliferation. pH measurements of the tumor tissue would be important as the pH can influence not only the delivery and uptake of drugs, but also affect the oxygenation of the tumor. Therefore, if it is determined that the pH of particular tumor is 7.2 and the uptake of the drug of choice is undesirably affected by a pH greater than 6.9, then the drug should be withheld and the pH changed. Cell proliferation can be measured with the aid of a beta radiation sensor able to monitor uptake of any radioactive tagged substance or ligands and provide information on cell kinetics and proliferation. If the uptake of a particular ligand which measures for cell proliferation is high (indicating active cell proliferation and therefore increased sensitivity), then the drug or radiation should be delivered.

It will be appreciated by those of skill in the art that at this time, specific dynamic changes and/or values of those changes occurring in pH or oxygenation of cell proliferation during and after treatment have not been definitively quantified (but which can now be established based on the dynamic monitoring provided by the present invention). Further, the pH, cell proliferation rate and schedule, and oxygenation can vary significantly from patient to patient, even within patient groups having the same type of tumor. Indeed, it is believed that this variability can account for the difference in response from patient to patient when treated with the same drug. Why should only 10, 20, or even 30% of patients respond to a drug that, according to *in vitro* data, should produce a tumor response of greater than 50%? Advantageously, the present invention will now allow data to be collected on specific values of for each monitored parameter or variable (preferably including pH, oxygen tension, and cell proliferation) during and following cytotoxic treatment. The collected data can be studied and a specific set of variables identified to affect a particular response. Armed with this information, a patient can be more effectively treated. Thus, the present

invention will now allow not only the establishment of specific variable information for evaluation, but, can also be used to direct and monitor the effects of treatment.

Thus, in a preferred embodiment, the present invention configures a tumor monitoring system with sensor elements designed to monitor one or more of tumor pH, oxygenation level, temperature, and cell proliferation. The cell proliferation can be measured presently by the use of a radiation sensor (which can also be used to verify the dose of radiation received at the tumor during radiation therapy). It is anticipated that other biochemical or biomolecules will be determined to be sensitive indicators of the vulnerability of the tumor for treatment and, thus, useful according to the present invention. The present invention can provide all these sensors in each tumor, gathering and transmitting the information in real time, to a computer containing an algorithm to process the information to determine if and how the patient is to be treated.

Turning now to **Figure 4**, an exemplary data analysis method is illustrated which evaluates and analyzes the data associated with the monitored parameters. As shown, the desirable values of selected physiological parameters (shown as at least three parameters A, B, and C) are identified or defined as they relate to the desired condition proximate to an active therapy (**Block 200**). The desirable values for each of the parameters may be input as a minima or maxima and may be cross-related to a particular treatment type. That is, one parameter, for discussion identified as parameter "C" (such as pH), may require or desire a certain minimum or maximum value to achieve maximum effectiveness particular to a certain treatment type (such as a particular chemotherapy or drug treatment). In contrast, another parameter, for discussion, identified as parameter "A" (such as oxygenation level) may have the same preferred value across all treatment regimes (typically a minimum value as a normal or an elevated oxygenation level is desirable). As such, if there is a minimum or maximum value at which therapy should not proceed, it is identified as a test criteria for data analysis just prior to the delivery into the subject of the treatment.

Similarly, a range of physiological parameter values particular to the parameter can be used as a basis for test criteria; for example, defining the levels associated with "elevated," "decreased" and "normal" can be input (**Block 210**). This criteria (as well as relative levels, population norms, or other indices of tumor behavior and treatment efficacy) can then be used to define test conditions corresponding to evaluation of tumor treatments (**Block 220**). That is, the test conditions can be any number of tests representing evaluation of the tumor and the treatment. As shown, the test conditions also test for abnormal values of the monitored parameters (**Block 231**). This can identify the malfunction of a sensor, sensor element, or other component of the monitoring system as well as identify a

potentially immediate need for medical evaluation. Other test conditions can include testing for elevated or decreased parameter values (**Blocks 232, 233**) respectively. Similarly, the presence of a clustering of "favorable conditions" represented by two of the parameters having increased or elevated parameter values and another having a decreased parameter value (**Block 235**) may represent a more favorable treatment period. For example, the presence of an elevated oxygenation level together with a period of increased cell proliferation and a decreased pH level may trigger a favorable treatment window. Of course, the clustering of just the two increased parameters can also be a test condition. In addition, one test condition can review the parameter values to determine variation from an expected value based on a predictive model (statistically relevant variation from a relative reaction or from a population norm) based on a point in time during or after active treatment (**Block 234**). A test condition which identifies whether the parameters meet the defined desirable values may also be helpful (**Block 236**). It may also be beneficial to have a test to determine if an expected data monitoring (local and/or remote) has been received or is missing (**Block 237**). This could indicate data corruption, file corruption, or even be used to automatically call the subject (such as with a programmed or recorded telephonic message) to notify them that a data transmission is needed.

In any event, the physiological data is periodically monitored (**Block 240**) and the data is compared to the test conditions/defined values (**Block 250**). An unfavorable active treatment time and a favorable active treatment time can then be identified (**Blocks 260, 261**), respectively. Of course, other evaluations and therapy decisions can also be made. The favorable test time can be identified by the test conditions/parameter values indicating a positive indicator (favorable condition or good progression). Of course, the data may also reflect a norm indicator (neutral condition), and a negative indicator (unfavorable condition or resistance to therapy). It is envisioned that a global network database or a regional database associated with each hospital or clinical site identifying the appropriate values can be pre-established to minimize the data input needed for a particular subject.

It will be understood that each block of the block diagrams (or block in the flowchart illustrations), and combinations of blocks in the flowchart illustrations or blocks in block diagram figures), can be implemented by computer program instructions. These computer program instructions may be loaded onto a computer or other programmable data processing apparatus to produce a machine, such that the instructions which execute on the computer or other programmable data processing apparatus create means for implementing the functions specified in the flowchart block or blocks. These computer program instructions may also be stored in a computer-readable memory that can direct a computer or other programmable data

processing apparatus to function in a particular manner, such that the instructions stored in the computer-readable memory produce an article of manufacture including instruction means which implement the function specified in the flowchart block or blocks. The computer program instructions may also be loaded onto a computer or other programmable data processing apparatus to cause a series of operational steps to be performed on the computer or other programmable apparatus to produce computer implemented process such that the instructions which execute on the computer or other programmable apparatus provide steps for implementing the functions specified in the flowchart block or blocks and/or block diagrams.

Accordingly, blocks of the block diagrams or in a flowchart illustration support combinations of means for performing the specified functions and program instruction means for performing the specified functions. It will also be understood that each block of the block diagram or flowchart illustrations, and combinations of blocks in the block diagrams or flowchart illustrations, can be implemented by special purpose hardware-based computer systems which perform the specified functions or steps, or combinations of special purpose hardware and computer instructions.

Although the present invention will likely provide additional basis for establishing more definitive numbers or values for monitored tumor physiological parameters, the following parameters and levels and indicators are provided as suitable for establishing test criteria associated with treatment or tumor condition. Conventional treatments use combination therapies such as temperature and radiation (tumor heated twice a week while irradiating every day).

Temperature

One approach to the treatment of large unresectable tumors is the use of radiation and thermal treatment. Typically, in such instances, the tumor is irradiated daily and heated twice per week following the daily radiation treatment. The temperature range preferred to achieve an increased, and hopefully maximum, cell kill is between about 42-43.5 °C. This temperature is then preferably maintained for about 20 minutes. The temperature is monitored closely to minimize the effects on the surrounding normal tissues and to assure that the same temperature is substantially homogeneously obtained throughout the tumor. This treatment technique is utilized and found to be effective for primary tumors from a number of tumor sites, including, but not limited to, the lungs, the prostate, the breasts, melanoma, the pancreas, and the pelvis. Thus, the present invention can provide an easy and effective thermal monitoring means by which temperature can be monitored, the thermal monitoring can prove especially suitable for externally inaccessible

tumors or for tumors located deep within the body, which are not easily monitored by conventional means.

Level of Oxygenation

The oxygenation level need to overcome radiation and or chemotherapy resistance has not been definitively established on dynamic systems as noted above. That is because, the precise changes which occur during treatment have not been quantified and therefore it is difficult to predict what definitive value may ultimately be established as necessary to overcome radioresistance now that dynamic monitoring protocols are available. This information will be obtained upon clinical applications of the proposed invention along with specific correlation with treatments and responses. Ultimately, lower oxygen tension may be found to be effective for treatments and that a normal or elevated oxygenation is not required for successful treatment. Nonetheless, the current preferred treatment approach is to achieve at least as normal a level as possible (and not to deliver during decreased oxygenation periods). Accordingly, for reference, the term "elevated" can be described as levels above 52 mm Hg. The term "normal" can be described as levels from about 50-52mm Hg. While the term "decreased" can be described as levels at or below 49 mm Hg, and more preferably, below about 40 mm Hg. It should be noted that oxygen is important for most, if not all tumor types, and is not specific to one type of tumor (although a particular level may be more suitable for treatment of one type). Further, *in situ* sensors according to the present invention can be positioned at different positions within the tumor to monitor the distribution of oxygen. If a significant difference (or delta) is detected, an attempt can be made to increase the oxygen levels to a sufficient level across the tumor.

Accordingly, the radiation or chemotherapy treatment can be withheld and given only when the oxygenation level approaches a minimum of about 50mmHg or is within a range determined to be appropriate for that patient (based on a relative response and/ or absolute response data).

Cell Proliferation

As noted above, cell proliferation is an important property of malignant tumors which can effect outcome. A knowledge of the time during which the tumor cells are proliferating is important in order to achieve a greater cell kill, and in turn, a greater response to therapy and an improved outcome. The degree of cell proliferation is related to the number of cells, which are cycling. Thus, if a ligand associated with cell proliferation is tagged, it will be incorporated into cycling cells and reveal itself as increased radioactivity within the tumor. Under normal or

quiescent conditions, only about 2-5% of cells are typically cycling. This quantity will increase generally by an order of magnitude to 20-25% in a moderate or highly proliferative state. The difference in uptake of the radioactive material will be noticeable and can be correlated to periods of increased cell proliferation. The time during which this increased proliferation is not readily known and has not been readily identifiable. The time during which cell proliferation occurs may vary with the specific tumor type, as well as the rate of proliferation itself (the time it takes to double the population).

Tumor pH

The pH of tumors has been found to be lower (more acidic) than the pH associated with normal tissue. The precise pH or range of pH needed for maximum effect is not known, nor have the fluctuations encountered during treatment been quantified as noted above. The impact of information regarding pH can be more complicated than that oxygen since pH may effect oxygen level, drug uptake, and cell proliferation. In addition, surrounding normal tissue can also effect the tumor pH. At present, it appears that a more acidic environment (pH of between about 6.8-7.0) may be preferably for treating malignancies. This is based on *in vitro* data which indicates that at least one drug, adriamycin, is more effective at low pH. As also noted above, the difference in pH between normal and malignant cells can be narrow (about 0.4 units) and therefore may indicate that there is a narrow treatment range at which drugs and radiation are more effective. As noted above, the present invention can now determine, in real time, the changes that occur during and after cytotoxic therapy.

Radiation

Radiation monitoring can be used to identify cell proliferation above (typically beta radiation). Radiation sensors can also be used to verify irradiation doses delivered during photon irradiation treatment (typically in the range of between about 3000-6000 cG). Thus, use of a radiation monitor during real time delivery can help control a more precise delivery dose of gamma radiation to the tumor site (distribution of dose within the tumor following photon irradiation or verification of calculated dose, especially with high dose conformal therapy). ß radiation monitors can also monitor radioactively labeled compounds to monitor drug uptake and utilization, blood flow the tumor, sensitivity to specific drugs, drug distribution in various organs (as well as cell proliferation discussed above).

In summary, a number of tumor (and proximate normal cell) parameters can be monitored, each of which can provide information material to the treatment and condition of a tumor in a subject. Individual parameter combinations thereof, and

biomolecular tumor parameters yet to be identified may also be monitored according to the present invention.

Biotelemetry and Implantable Sensors

It will be appreciated by one of skill in the art that when a foreign object is implanted into the body, a series of host responses occur: 1) deposition of blood plasma proteins, 2) fibrin formation, 3) assault by immune cells and proteins, 4) attack by inflammatory cells, and 5) formation of a cellular capsule around the object (Reichert et al., 1992). Therefore, it is important that the materials used in an implanted device address this host response. Much is known about the implantation of sensor systems. Kapton® polymers have been shown to be relatively benign when used as a sensor substrate (Lindner et al., 1993). Pacemaker companies frequently use titanium cases with medical grade epoxies and silicone rubber to encapsulate the external lead connections (Webster, 1995). Implantable glucose sensors have been constructed using polyethylene cases covered in Dacron velour, with the sensor surfaces being coated with a variety of bioprotective membranes (Gilligan et al., 1994). (These units were wet sterilized in 0.05% thimerosal for 24 hours before being implanted and tested *in vivo* for up to three months.) A more common method used for sterilizing implant devices is gas sterilization at temperatures of 115° C to 120° C for 20 minutes.

Early researchers used discrete components to implement simple oscillator circuits for implantable sensors (Mackay, 1995). In recent years, the focus has been on miniaturization, using hybrid and integrated circuits for the electronic portions of the systems. Because the demand for "high-tech" biotelemetry systems in the past has been small, few suppliers have invested resources into developing state-of-the-art systems and devices. Most of this development has been performed at academic institutions around the world, with an emphasis on creating smaller, more-efficient telemetry and telemetry-like devices with increased functionality.

Integrated circuit (IC) technology has been used significantly for biotelemetry device electronics throughout the past two decades. In the mid 1970s, IC usage was made feasible through the use of hybrid technology. This technology enabled engineers to construct telemetry devices by interconnecting commercially available ICs, simple custom ICs, and other discrete components, on ceramic substrates through the used of thick- or thin- film technologies (Fryer et al., 1973; Deutsch, 1979; Gschwend et al., 1979; Donald et al., 1981). Perhaps the best example of this technology is a unit perfected at NASA Ames (Hines et al., 1995). NASA uses a carrier of 455 kHz and digital PCM. The implanted unit is fabricated using hybrid technology and monitors pH, heart rate, temperature, and battery voltage. Its current

consumption is less than 120 microamps drawn from a 0.75 A-hr lithium battery. The battery lifetime is 6 - 9 months. The unit is packaged in a custom-manufactured, disk-shaped ceramic package, approximately 3.0 cm in diameter occupying a volume of 20 cc. The telemetry link has an acquisition range 12 to 24 inches.

As the microfabrication processes improved, telemetry units could be fabricated on individual silicon substrates only millimeters in length and width. Recently, biotelemetry systems have been appearing with custom integrated circuits as a major component (Oshima et al., 1987; Williams et al., 1994; Wouters et al., 1994; Akin et al., 1995). In a typical example (Puers et al., 1993), an intelligent 4-channel unit was designed and fabricated for animal husbandry studies. The electronics used for this device were created on a 4.7 x 7.1 mm^2 silicon substrate and included both analog and digital signal conditioning electronics to process the incoming signals, transmit them accordingly, and direct power to the appropriate sub-circuits when needed. As with most IC based transmitters, a few external devices were required for operation, including capacitors and crystals for driving the IC oscillators, accelerometer and temperature sensors, and resistors and switches to set gains and identification codes. It is important to note that such additional components can be undesirable, since they can add to the physical size of the electronics and increase the overhead involved in fabrication. They do, however, give the user/designer more flexibility in modifying circuit operation.

A novel implantable telemetry system was recently under development at North Carolina State University (Fernald et al., 1991 and 1992). The system was intended for rapid-prototyping applications, and was designed such that a non-engineering researcher could construct a customized implant device with minimal effort. The system consisted of two core intelligent integrated circuits, a microprocessor/telemetry chip and a data acquisition chip that could be stacked upon one another and fully interconnected with less than ten bus wires. Although the data acquisition chip provided eight input channels, additional channels could be attained by stacking additional such chips and attaching them to the bus lines in a daisy-chain manner. The microprocessor was fully programmable (both before and after implantation) and possessed an instruction set suitable for processing biological signals. The system was intended for a variety of transducers with varying bandwidths. As a consequence of the serial bus architecture, the system throughput was limited to an aggregate bandwidth of 20 kHz, suitable for most applications.

Researchers have long sought methods to eliminate the batteries in implanted devices (Hansen et al., 1982). Inductive power coupling has received attention in recent years. One research group (Benedetti, 1995) developed an inductively powered implant with four channels for measuring pressure and EMG. The sampling rate was

200 Hz/channel; its size, 15 x 19 x 86 mm^3; and its weight, 55 g (40 g is the housing). The implant was mounted in a gold-plated brass housing. Surface mounted components were attached to stackable printed circuit boards. The internal power sources were + 3 V and - 3 V, derived from a power carrier frequency of 27.1 MHz. Current consumption was 6 mA. The transmission/coupling range was 30 - 70 mm. The telemetry links were sampled FM with a frequency range of 36 kHz - 120 kHz.

A second example system incorporating inductive powering was designed for orthopedic measurements (Graichen et al., 1991 and 1995). This unit implemented eight telemetry channels (6 for strain sensing, one for temperature, and one for power supply voltage). The electronics module was a thick-film hybrid substrate with custom IC and discrete components. The substrate was encapsulated in a titanium cylinder measuring 8 mm in diameter and 27 mm high. The telemetry links operates using pulse-interval modulation with a carrier frequency of 150 MHz. The operating range is 20 cm. The implant is inductively powered through a 4 kHz coupling channel.

Inductive powering is also finding applications in cardiovascular and neural studies. A novel 3D power steering scheme has been proposed for high-data rate cardiac mapping studies (Mueller et al., 1995). Researchers have also implemented inductive powering in some telemetry-controlled neural stimulators. Their size has been greatly reduced, allowing them to be injected into tissue through a hypodermic needle. Two such devices have been reported by researchers at the University of Michigan (Akin et al., 1990) and the Illinois Institute of Technology (Loeb et al., 1991). Both systems rely on micro coils and magnetic induction to power the devices, thus eliminating the size and weight associated with batteries. The inductive links were also modulated to convey command information to the implants. Further reduction in size was achieved through CMOS integrated circuit technology. Both research groups proposed incorporating reverse communication capabilities, so that the implanted devices can also perform telemetry monitoring functions (Nardin et al., 1995).

Commercial manufacturers have been successful in building and marketing a variety of models. These systems only have a few channels and are tailored for animal research. For example, Data Sciences International (St. Paul, Minnesota) offers a number of models. Their systems use pulse-interval modulation, a low power consuming technique. However, their systems typically use a single carrier frequency per channel, limiting the number of channels that might be implemented. The low input impedance of their electronics also limits the possibility of including pH and other ion-selective sensors. Another limiting factor in the Data Sciences system is its unique, proprietary signal encoding, transmission, and receiver units. Therefore, the possibility of expanding beyond four channels (their upper limit) is quite unlikely.

Coupled with the fact that these units are larger than needed and that the upper limit is 35 °C for their temperature sensors, Data Sciences units are not appropriate for this application.

Telemetry units from Mini Mitter (Sun River, Oregon) are very small in size (XM-FH series - 9.0 mm (dia.) x 15 mm; VM-FH series - 12 mm (dia.) x 19 mm). They use the pulse interval modulation transmission mode to achieve very low power operation. However, they monitor only a single channel. Therefore, stacking several single channel transmitters to build a multi-channel device could make the combined size unacceptable. Small button-type batteries are used and are easy to replace. These units are attractive for single channel applications.

Biotelemetrics (Boca Raton, Florida) builds transmitters whose carrier frequency is adjustable, which makes it possible to stack a series of single channel transmitters to make a multi-channel unit. The size of a typical unit is approximately 2.5 mm x 7.5 mm x 10 mm. The transmitters can be turned on and off periodically to reduce the power consumption. The electronics exhibits a high input impedance which enables the unit to be connected to any kind of sensor (*e.g.*, thermistors, pH sensors, and other ion-selective sensors).

Konigsberg Instruments (Pasadena, California) offers four- and eight-channel implants for measuring temperature and biopotential signals (such as EEG, ECG, and EMG) with a bandwidth up to 1 kHz. The units range in size from the smallest 1.0 cm x 1.5 cm x 3.3 cm to the largest 5.1 cm x 2.3 cm x 1.5 cm. The units are battery powered and the battery life ranges from five to 20 months. An RF switch is included to turn the battery on and off. The transmit range is typically 3 - 5 m. Multichannel amplifier units are also available to receive the transmissions from the implants and relay them to a remote base station. Several other small companies make biotelemetry devices (Bio-Sentry, CME Telemetrix, Coulbourn, MIE Medical, Micro Probe, Telefactor, and Transkinetics), but they are not implantable or are single-channel units (Biotelemetry Page, 1997).

Button battery cells have been available for nearly three decades, and were extensively used in hearing-aid devices. The most commonly used cells of this type are available in two chemistries - zinc-mercury oxide and zinc-silver oxide. The primary functional differences between the two are as follows: (1) zinc-mercury oxide exhibits a flatter discharge voltage characteristic over time, (2) zinc-mercury oxide responds better to momentary high-power demands (low internal resistance), (3) zinc-silver oxide has a higher output voltage, specifically 1.5 to 1.6 V, versus 1.35 V from zinc-mercury oxide, and (4) the volumetric energy density of zinc-silver (monovalent) is greater ranging 400-550 Wh/cm^3. The service capacity of these cells is typically near 100 mA-hours.

Another alternative to these cell types are the recent lithium-anode based cells. These cells are desirable because their output voltages (near the 3 volts needed for ICs) are typically twice that of zinc-anode cells. Another notable difference is that lithium cells are typically available in flat packages and are appropriately termed "coin-cells." From a volumetric standpoint, the energy densities of most lithium-based cells compare favorably to zinc-based cells. For example, lithium-iodine cells exhibit a 2.8 V output with a high energy density of approximately 1,000 Wh/cm^3. Pacemakers have used lithium cells since the 1970s.

Preferred Tumor Monitoring Devices

Some preferred sensor embodiments of the present invention are illustrated at **Figures 5, 6A, 8, 9,** and **22**. Generally described, the *in situ* sensor units **50** of the present invention are configured to be one of implantable or injectable into the subject. **Figures 5, 6, 21,** and **22** illustrate preferred implantable embodiments, while **Figure 8** illustrates an injectable embodiment. **Figure 9** illustrates a hybrid sensor unit **50''** having both an implantable satellite sensor body **50S** and associated injectable dependent sensor bodies **50D**. Each of the sensor units of the present invention are powered either by a battery (**Figure 5**), or, and more preferably, is inductively powered (**Figures 6A, 8,** and **9**). Each of the (implantable or injectable) sensor unit bodies is hermetically sealed with biocompatible materials and sterilized by methods well known to those of skill in the art.

As shown in **Figure 5**, the sensor unit **50'** is configured with at least one sensor element **51**. The sensor element **51** shown in **Figure 5** is a thermistor. More preferably, as shown in **Figure 6a**, the sensor unit **50** comprises a plurality of sensor elements **51a-51e**, which are preferably configured to monitor one or more of temperature, radiation, oxygen, and pH. Suitable discrete pH, radiation, and temperature elements **51a-51e** are known to those of skill in the art. The preferred temperature sensor type is a thermistor. The preferred radiation sensors are well known such as MOSFET (metal oxide semiconductor field effect transistor) based designs. Preferred self-calibrating oxygen and combination oxygen/pH sensor embodiments will be discussed further below.

The temperature sensor element for the present invention is configured to operate in the temperature range of about 35° C to 45° C with an accuracy of about 0.1° C. Size is of major importance since the entire implantable device should be minimally invasive. Preferably, the entire implantable sensor unit is sized to be less than about 1.0 cm^3. Further, the sensor units **50, 50', 50''** of the tumor monitoring system **10** are configured to operate even when exposed to a radiation field. That is, the sensor unit **50, 50', 50''** do not necessarily have to function while the radiation is being administered to the tumor, but they preferably function immediately afterward.

The sensor unit **50, 50', 50''** is thus configured to respond quickly (within a few seconds) after radiation administration. In a preferred embodiment, as shown in **Figure 8**, the sensor unit **50''** is sized and configured such that it can be placed on the tip of an insertion probe and injected via a large bore canula such as via image guide placement into position.

Referring now to **Figures 6A** and **6B**, a preferred embodiment of a sensor unit **50** is shown. The sensor unit **50** is configured with a primary body portion **50B** and a plurality of arm portions **50A** extending outwardly therefrom. As shown in **Figure 6B**, the arms **50A** have a thin planar profile. Preferably, the arms **50A** are formed of a flexible biocompatible substrate material such as a polyimide (like Kapton®, a polyimide material). At least one sensor element **51** is positioned on each arm **50A**, preferably at a distal portion (away from the primary body **50B**). A separate channel **151** electrically connects the sensor element **51** to the electronic operating circuitry **125** positioned on the primary body **50B**. Of course, a plurality of sensor elements **51** can be positioned on each arm, each with a separate electrical communication channel **151**. Preferably, each channel is defined by a pair of leads (the sensor O_2 may have greater than two (2) leads) formed by metal vapor deposition onto the top surface of the flexible substrate.

As is also illustrated by **Figures 6A** and **6B**, the transmitter coil **58** is substantially circumferentially layered to surround the electronics **125**. The electronic circuitry **125** includes at least one, and preferably a plurality, of fixed resistors **125R** for signal data reference as will be discussed further below.

As shown in **Figure 6B**, a biocompatible coating **160** is applied (to preferably encasulate, and more preferably, hermetically seal) to the exterior of the sensor unit **50**. Surface mounted electrical components can also be located on the bottom surface of primary body **50B**, with interconnection being made by plated through vias (a common method used in flexible printed circuit board technology). Advantageously, this multi-arm configuration can provide increased regional data to allow for more informal analysis of the tumor. As discussed above, the multiple sensor elements **51** can contact different locations within (penetrate at different depths) and/or wrap to contact different exterior perimeter locations along the tumor. Alternatively, one or more arms can be attached to normal tissue to provide information regarding the status of same. In any event, the sensor arms **50A** are preferably configured with attachment means **150** to secure their position in the subject. For example, sensor element **51A** illustrates an aperture **150** formed in a distal position of the substrate to allow a suture to attach it in position. Alternatively, sensor element **51b** illustrates a barbed outer surface **150'**.

Figures 7, 8A, and 8B illustrate a sensor unit 50'' which is cylindrically shaped and sized for injection, *e.g.*, an injectable sensor unit **50I**. In this embodiment, a PCB or IC chip **125p** is oriented to extend a small distance along a length of the sensor body. The coil **58** also cylindrically extends to surround a portion of the PCB or IC **125**. In the embodiment shown, the PCB is a substrate (preferably a flexible substrate) which extends a distance outside the coil **58** (for an overall length which is less than about 0.5 inches). Of course, with the use of an IC configuration, this size can be further reduced. In addition, the IC or PCB can be configured and sized to extend substantially the same distance as the coil **58**. The sensor body can be configured to hold a single channel (*i.e.*, one sensor element for a PCB version having a width of about 3mm) or multi-channel (multiple elements, with each channel layed side by side, and typically wider than the single channel version). The tip **125T** of the sensor unit **50I** can be configured with a rounded or pointed edge to help facilitate entry into the tumor tissue. Again, the entire sensor body is encapsulated with a biocompatible material and sterilized for medical applications.

Preferably, both the injectable and implantable versions **50I**, **50**, respectively, of the sensor units of the present invention, such as those shown in **Figures 6** and **7**, are inductively powered. That is, the monitoring system is configured to act as a transformer (with one coil on the surface of the patient's body and the second within the monitor) to couple and power the internally disposed sensors, as is well known to those of skill in the art and discussed briefly above. As such, the *in situ* sensor units **50, 50', 50", 50"'** are self-contained, and have a sufficiently long service life in the body to provide clinically useful chronic information for episodic or chronic treatment decisions, and can be miniaturized without requiring catheters or externally connected wire leads into the sensors and out of the body.

Alternatively to the separate copper wire wrapped coil conventionally used to form the coil **58**, the coil **58** can be integrated into the circuit board itself via a ferrite substrate (a flux concentrator). Further, the circuit board **125p** and its associated electrical components can be configured as a miniaturized chip which allows the coil **58** to be similarly miniaturized. Note, however, that the signal is typically proportional to the area of the coil and, as the area of the device decreases, the signal strength associated with the coil **58** on or around the device can decrease.

It will be appreciated that to further miniaturize the device, the temperature sensor resonant element can be configured as a positive temperature coefficient (PTC) (typically ceramic). Although most conventional devices employ NTC (negative temperature coefficient) versions, for the instant application, the PTC may be advantageous.

Figure 9 illustrates a hybrid sensor unit **50'''** version of the inductively powered implantable and injectable sensor units **50, 50I** described above which allows for miniaturized sensor element bodies and useful signal strength at transmission. As shown this sensor unit **50'''** embodiment includes a satellite sensor unit **50S** with the IC or externally communicating electronics **125** thereon and a plurality of dependent sensor units **50D**. The dependent sensor units **50D** are inductively coupled to the satellite sensor unit **50S** which is, in turn, inductively powered and coupled to the external system. Further, the dependent sensor units **50D** are telemetrically connected **60I** to the satellite sensor units **50I**, which is telemetrically connected **60** to the external receiver **75**. Because the dependent sensor units **50D** are locally positioned relative to the satellite sensor unit **50S**, the signal strength demands are reduced, thereby allowing the injectable sized dependent sensor units **50D** to be further reduced in size. Preferably, each dependent sensor units $\mathbf{50D_i}$ can be electronically encoded or identified or positionally related to a particular channel or port within the satellite sensor unit **50S** to maintain relative (if not absolute) positional information for consistent data analysis of the transmitted sensor data for the monitoring system **10**.

Figure 19A illustrates another embodiment of the present invention, at least one wherein the tumor monitoring system **10'''** employs a plurality of sensor units **50**. That is, at least one sensor unit **50** is positioned at a different (separate) tumor site as shown. This multi-sensor unit tumor system **10'''** can result in more regional specific information to adjust treatment as necessary to effective in each tumor site of concern. Preferably, the multi-sensor monitoring system **10'''** will configure each separate sensor unit **50, 50", 50"'** to be electronically identifiable to maintain data integrity and correlation to the tumor site/particular location. This can be provided by configuring the receiver **75** and the separate sensor units **50** (**50I** and **50S/50D**) with port communication protocols to identify and/or maintain the relative order of transmittal to the location or position of the particular sensor unit **50** within the body (*i.e.*, channel one for "sensor 1," channel 2 for "sensor 2," each alphanumeric identifier being manually or programmably set at insertion or position onto the tumor in relation to its left to right or up to down position to a relational axis). As the receiver **75** should be positioned proximate to the sensor unit coil **58** (typically about 30 cm) for proper data transmission, it is preferred that the receiver **75** be configured to move to overlay the appropriate sensor unit during transmission (indicated by the arrow and dotted line movement of the receiver in **Figure 19A**) and it is also preferred that the receiver **75** be programmed to recognize the order of sensor unit transmission to assure data integrity. Of course, two receivers can be used, one for each sensor unit location. This may be especially appropriate for non-clinical use, such as at a patient's home wherein a patient interactive system may be needed. Thus, a dual

receiver configuration, whereby a user can keep in position a portable receiver over each monitored tumor site, can be advantageous.

Of course, an external mark or indices of alignment to allow proper alignment may also be helpful (both in a single tumor/region sensor unit embodiment and a multi-sensor unit/spaced position embodiment). This can be a semi-permanent physical mark **175** made on the skin and/or other infrared or photogrammetric position readable or indication means which can cooperate with the receiver **75** (receiver loop) such that the receiver **75** can send out a position verification beam to facilitate proper alignment before/during transmission at the selected location.

For remote transmissions, the tumor monitoring systems of the instant invention are preferably configured to transmit information at a low or very low bandwidth. That is, the carrier bandwidth is preferably in the MHz range while the modulation frequency is more preferably at or below about 1kHz. This low bandwidth operation allows transmission of signal data received from the sensors across slow communication links such as modem and voice telephone connections. Preferably, the measured signal information is encoded into one of several time-based modulation schemes. The time-based encoding permits accurate data transmission across communication links that may convey amplitude information inaccurately and frequency information accurately, such as the voice telephone network. In addition, for home site non-clinical use tumor monitoring systems **10'**, the monitoring equipment is preferably small and relatively inexpensive or cost-effective to be set-up and operated at remote locations.

Of course, the low bandwidth operation is not required as the data from the sensor units **50, 50I, 50S** can be converted into essentially any number of suitable encoding or transmission schemes that are suitable for remote operations, as discussed above, such as substantially continuous or semi-continuous monitoring with a PC at the remote location and storing the data associated with same with time/date stamps so that a complete data set or data segment/record covering a period of hours, days, weeks, or longer can be gathered and transmitted to the central processing site over one or more discrete relatively short data transmitting sessions.

Of all of the major types of temperature sensors, typically the thermistor is by far the most sensitive. It has a fast response time and a high output for interfacing, and small devices are commercially available. The non-linear response is not critical over the small temperature range in which the sensor will function (typically less than about 10°). Although the interfacing circuits require a current source, the silicon overhead is only a few additional transistors. The device is considered fragile for industrial purposes, but should be amply rugged for this application. Sensor self-heating is reduced since the device operates in a limited temperature range and the

current can be small and need not be applied continuously. If a battery source is used, the sensor element is preferably insulated or positioned spatially away to reduce its exposure to heat attributed to the battery.

To validate a tumor sensor design, a single-channel, discrete-component, commercial telemetry unit was purchased (Mini Mitter, Inc., Model VM-FH) with externally mounted thermistor. An experiment was conducted at Triangle Radiation Oncology Services (TROS) by placing the thermistor and transmitter into an agar-gel phantom target, and heating the target in a hyperthermia treatment device (Thermotron RF-8) over the therapeutic range of 37°C to 45°C. **Figure 2A** illustrates the principle of operation of a hyperperthermia treatment with a Thermotron® device. An eight MHz RF signal is applied between the plates of the machine which causes ions between them to oscillate. These oscillations generate heat by friction, producing uniform self-heating of body tissue. The agar-gel phantom is approximately the size of a human torso and mimics the heating characteristics of body tissue. During treatment sessions with a patient, the skin surface temperature is always monitored. In addition, catheters are normally inserted through the skin surface into the tumor undergoing treatment and its vicinity. During treatment, thermocouple probes are inserted through these catheters to record tumor temperatures as the RF energy is applied. These catheters are left in place in the patient between treatment sessions and are frequently a source of discomfort and infection.

This experiment was designed for two purposes. First, the performance of the insulated thermistor was compared to that of a Thermotron® thermocouple, and secondly, to observe the heating effects of the Thermotron® device's RF energy on a bare, button-sized battery placed in the agar-gel. The experimental setup is illustrated in **Figure 11.** Two catheters **99** were inserted into the agar-gel phantom **101**: one positioned near the thermistor **99R** and the second near the battery **99B**. Thermotron® device thermocouple probes were inserted into the catheters and RF energy was applied to the agar-phantom to gradually sweep its temperature over the therapeutic range. The experiment was designed to be conducted over a 75-minute time period to ensure that the agar gel was heated uniformly.

The results of the experiment are presented in Table 1. The first two columns of Table 1 show the time progression of the experiment and the temperature reading from the Thermotron® device's instrument panel taken from thermocouple-1 (see **Figure 11**). This measurement was assumed to be correct and was used as the reference or "gold" standard. The third column shows the relative change in the temperature of thermocouple-1 from its initial value in the first row. The fourth row shows the relative change in the thermistor's readings at the same measurement times. Note the close correlation with the Thermotron® device's thermocouple readings.

The results of the button battery heating experiment are reported in the fifth column of Table 1. These data were recorded from a thermocouple-2 located near a button-sized battery placed in the agar-gel phantom. Note that the temperature near the battery increased to a larger extent as the RF energy of the Thermotron® device heated the agar-gel over the therapeutic range. While the temperature of thermocouple-1 near the thermistor increased by 8.8°C, the temperature of thermocouple-2 near the battery increased by 11.1°C. This indicates that any implant that is powered by a battery should be properly thermally insulated to minimize its impact on temperature sensors that are monitoring the environment of tumor cell populations.

TABLE 1. Agar-Gel Phantom Experimental Results.

Time (minutes)	Thermocouple-1 Temperature (°C)	Thermocouple-1 (T)	Thermistor (T)	Thermocouple-2 (T)
0	35.7	0	0	0
7	36.9	1.2	1.2	1.6
24	38.5	2.8	2.8	3.7
37	41.0	5.3	5.3	6.6
57	43.5	7.8	7.9	9.7
72	44.5	8.8	8.7	11.1

The next task was devoted to designing and building a 4-channel, discrete-component prototype circuit using breadboarding techniques. This circuit utilized four thermistors for temperature monitoring. A block diagram of the circuit is illustrated in **Figure 12**. Temperature increases were sensed by the four thermistors **51a-51d** in response to a corresponding reduction in resistance. A constant current source driving the thermistors **51a-51d** was used to measure the resistance. The amplifier **53** voltage output was proportional to the resistance change. A voltage to current converter **54** attached to the amplifier **53** was used to charge a timing capacitor **56**. The time period for the voltage on the timing capacitor to reach a threshold was proportional to the change in resistance in the thermistor **51e**, and hence proportional to the temperature change at the thermistor's surface. **Figures 13** and **14A-14C** show suitable operational design for sensor circuits. When the capacitor voltage reaches a preset threshold, the transmitter **157** sends a signal burst at 1.0 MHz to the coil **58**. At the same time, the threshold detection circuit **158** discharges the capacitor **56**. At the end of the signal burst, the capacitor **56** is allowed to again begin charging toward the threshold value. If the amplifier **53** voltage is high, a large current is dumped into the capacitor **56** leading to a short charging time interval. If the voltage on the amplifier output is zero, then no current is dumped into the timing capacitor **56**. In this case, a

small current source was included to ensure that the device is operating properly. This small current source forced the transmitter **157** to send out signal bursts at a large time interval for testing purposes. Longer time intervals indicate lower temperature measurements, while shorter ones indicate higher temperatures.

The clock, counter, and control logic **155, 156** serve to multiplex the four thermistors **51a-d** over the biotelemetry channel in a round-robin fashion. A modified AM radio receiver attached to a laptop PC running LabVIEW® software (National Instruments, Inc., Austin, TX) was used to detect the transmitter bursts. Water bath experiments were used to validate the operation of the implant design. The range of the telemetry link was about 30 cm.

Following the design and construction of the discrete-component breadboard, a surface-mount (SMT) unit was designed and constructed to reduce the size. The circuit of **Figure 12** was refined and a double-sided, 2.5 x 3.5 inch, printed-circuit (PCB) was fabricated. Low profile SMT components were used. The power consumed was 4.5 W from a 3.0 V battery. The transmitting coil **58** (13.5 mm in diameter) was formed with 25 turns of #38 AWG copper wire, producing a range of 30 cm. Four thermistors **51a-d** were attached to the device and the water bath experiments were repeated. Results were similar to the earlier experiments verifying the functionality of the system.

Following the successful SMT experiments, a first-generation integrated circuit (IC) test chip was designed. Its purpose was to demonstrate that the operating concepts adopted for the SMT unit can be adapted for integrated -circuit technology. **Figures 15** and **16** depict the functional blocks of the IC design and its chip layout. The circuit design was first refined and simulated using SPICE. Then an IC layout was performed using standard cell technology. The circuit was specifically designed to minimize its susceptibility to latch-up. The test chip was implemented using the MOSIS fabrication service (2.0 micron, CMOS, n-well, low-noise, Orbit analog process, tiny-chip frame). Several internal test points were inserted to allow complete testing of the IC subcircuits. Four ICs delivered in dual-in-line (DIP) packages were mounted in an IC prototype unit constructed using a small PCB (1.5 x 4.0 cm). All four ICs were subjected to benchtop functional testing and performed as expected.

After passing the functional tests, the test chips were exposed to a series of radiation and thermal tests. First the units were thermally tested using a temperature-controlled water bath as shown in **Figures 17A** and **17B**. The IC prototype unit used seven channels for sensor data. Four of the channels were connected to thermistors and the remaining three were connected to fixed resistors. **Figure 17A** illustrates that the thermistors caused the channel pulse width to vary by approximately 0.03 ms per 0.1° C while, as shown in **Figure 17B**, the fixed resistor channels varied by about

0.003 ms per 0.1° C. These results are well within the accuracy specifications for tumor sensors according to the present invention.

Next the units were exposed to radiation using the cancer treatment facilities of Triangle Radiation Oncology Services (TROS) located at Rex Hospital in Raleigh, NC. A series of 400 cGy radiation doses were delivered with a Varian Clinac 4/80 at a source to surface distance of 80 cm and a dose rate of 1.2 Gy/min. The IC prototypes were not powered during exposure, simulating one clinical environment in which the implants can be employed. The results of the radiation exposure tests are displayed in **Figures 18A** and **18B**. Note that the thermistor and fixed resistor channel pulse widths change by approximately 0.0015 ms per Gy, which translates to approximately 0.005°C per Gy. Given that a patient is not typically exposed to more than 8000 cGy, the impact of radiation is less than 0.4°C, which can be corrected by signal processing as described below.

The thermistor and fixed resistor data in **Figures 18A** and **18B** suggest that the increase in pulse width during exposure to radiation is due to changes in the active transistor parameters of the IC. These parameter changes are expected based on the experience of many researchers in the effects of radiation upon microelectronic circuits (NPS, 1997). Therefore, the IC device can be considered as a sensor for the radiation exposure.

Accordingly, a fixed resistor channel can be used to measure total exposure. From calibration data for each implant during manufacture, the initial pulse width for the fixed resistor channel will be known. From statistical data obtained about the behavior of the ICs under radiation exposure (data similar to **Figures 17A** and **17B**), the slope of the curve will be known. Therefore, real-time measurements from the fixed resistor channel can be compensated to account for the variation based on the reference fixed resistor and known calibration data to give an accurate indication of the radiation exposure history for the implant. Using this total exposure computation, the temperature reading from the thermistor channels can be corrected mathematically to give accurate temperature reading at any radiation exposure level. That is, radiation damage or exposure can cause IC drift, and temperature drift. This is compared to three parameters: a known fixed resistor value which is constant, a temperature sensor value which varies only in response to temperature, and the IC which is affected by both (thermal and radiation). Use of the calibration data established at set-up (or in the factory) can calibrate the signal data based on the number of known parameters to determine the radiation based drift and adjust for same. This drift is correctable as the dose of radiation is well within the drift adjustment as indicated by the **Figures 17** and **18**. In operation, a computer means can computationally perform the correction based on the data it receives from one or more fixed resistors.

Accordingly, it is preferred that at least one fixed resistor **125R** be used in the operating circuitry of the sensor, and preferably a plurality of fixed resistors. **Figure 14B** illustrates one fixed resistor channel (one reference) and four active monitoring channels. In one embodiment, the sensor unit **50** includes three resistors, one is substantially invariant with temperature or radiation (the fixed resistor **125R**), one changes with temperature (a thermistor), and one changes with both temperature and radiation (typically the MOSFET's in the chip have a resistance that changes with both). The thermistor has an associated measured temperature dependent curve. The fixed resistor can be used to correct the bias on the MOSFET"S (adjust or compensate for their drift due to radiation exposure/damage). The computer can give a corrected reading such as a temperature profile.

During normal operating conditions, the implant device may be powered down when radiation (high dose-rate gamma, thermal RF and microwave, or ultrasound) is applied to the patient. A series of tests were conducted to determine the effects of exposure/energy challenge events from exemplary treatment sources at Triangle Radiation Oncology Services (TROS). First, 8 MHz energy (Thermotron RF-8) at levels well above those used in treating patients was applied to the device in both its powered-down and powered-up states. Next, the tests were repeated for gamma radiation using a Varian Clinac 4/80. Finally, the tests were again repeated using microwave (915 MHz) energy from a Clini Therm surface tumor heating instrument. In all cases, the device was not damaged by the energy challenge tests, and continued to make accurate temperature measurements after the conclusion of the tests. All test were conducted on the same implant device so that the cumulative effect of the challenge tests were negative.

In order to assess biosurvivability and biocompatibility, several mock implant devices were fabricated using materials that are similar to the preferred embodiments of the sensor units described above. The overall scheme for fabricating a mock implant is highlighted in **Figure 5**. The substrate **120** can be fabricated using five-mil flexible Kapton® polyimide material covered by a 25 micron copper layer. The metal layer **122** is patterned using photolithography into the wiring harness for a simple oscillator circuit. Next, an insulating layer of polyimide can be deposited and patterned to open conducting vias to the metal traces. Then surface mount electrical components **125** are placed and soldered to the substrate. Next, a thermistor **51** is connected to the end branch of the implant substrate as shown in **Figure 5**. Then a coil of antenna wire **58** is mounted with the IC and/or SMT components **125** as illustrated in the figure. Finally, a lithium coin-shaped battery **52** is attached to the substrate **120**. The battery **52** is first affixed to the substrate in the position shown in **Figure 5**. The end flap **129** (the circle that contains the second battery connection) is

then folded over the battery and attached using conducting silver epoxy. The entire device is then encapsulated in a biocompatible material such as a thin layer of silastic and/or medical-grade silicone to protect it from the biological environment during implant.

Additional features can also be included in sensor units **50, 50', 50'', 50'''** based upon the specification of the user interface. For example, the ability to turn the battery on and off with an externally applied RF signal can be included in an IC (chip) design. Another feature can be the inclusion of pH sensor interface electronics. The pH sensors will preferably be implemented on a biocompatible, flexible substrate such as the Kapton® substrate shown in **Figure 10A** (Cosofret, 1995). This design is compatible with the Kapton® substrate shown in **Figure 5**.

In one preferred embodiment, the present invention employs self-calibrating oxygen, pH, or combination oxygen/pH sensors. The operating principle of the *in situ, in vivo* self-calibrating chronically implanted sensor systems **200, 201, 300** is based on water electrolysis at noble metal electrodes as shown in **Figures 20** and **22**. Oxygen or hydrogen can be evolved by the electrolysis of water by applying a current through a generating electrode ("GE") **227** and counter-generating electrode ("GE'") **227'** for a certain period. Accumulation of these dissolved gas molecules at the GE **227**, in turn, rapidly establishes a microenvironment of oxygen saturation or hydrogen saturation in close proximity to the microsensor. A two-point calibration procedure for the oxygen sensor unit **200** can then be performed, with the high point calibration being established in an oxygen-saturated phase, and the low point calibration in an oxygen-depleted phase that is produced by saturating the microenvironment with hydrogen. These transient perturbations of the microenvironment are expected to equilibrate rapidly with the surrounding medium (tissue). With this *in situ, in vivo* self-calibration sensor units **200, 201, 300** periodic sensor calibration can be performed to check the operability and biosurvivability of a chronically implanted device.

It is preferred that the self-calibrating sensor units **200, 201, 300** be configured with the following operational and physical specifications:

(1) Dynamic range:
   (a) 0-760 mm Hg with at least 10 mm Hg resolution (for oxygen tension)
   and/or
   (b) pH 5.0 - 8.0 with pH resolution of about 0.1;
(2) Concurrent operation during hyperthermia treatment sessions; and
(3) Minimum 4-6 week (preferably 6 week or 1.5 month) period of operation and more preferably at least a 3 month period of operation.

The water electrolysis method can be extended to perform a one point, *in situ, in vivo* calibration of an implanted pH sensor unit **201 (Figure 10B)** as well. A micro pH sensor unit **201** that is surrounded by a generating electrode will experience a titrating pH microenvironment during water electrolysis. If one repeatedly drives the electrolysis current forward and backward through the generating electrode, the highest slope in the time response of the pH sensor will occur at the moment of neutral pH (pH 7.0). Thus, a one-point calibration at neutral pH can be performed during water electrolysis by checking the first derivative of sensor response during titration. The functionality of similar pH titrating microdevices has been demonstrated for a pH-static enzyme sensor or buffer capacity sensor (Olthuis, 1992). This prior work strongly supports the feasibility of one point pH calibration as an option in tumor monitoring applications.

Previously, polarographic micro-oxygen sensors were fabricated on flexible Kapton® material. The basic electrochemical three-electrode cell configuration shown in **Figure 21** was adopted to avoid current flow and minimize surface material consumption in a micro-reference electrode. All electrodes were designed to be geometrically symmetric to assure diffusional mass transport of electrochemical species in all radial directions.

Two different designs were considered -- one with rectangular bands and another with concentric circles. The design with concentric circles gave better performance, which can be explained theoretically. The noise at an electrode-electrolyte interface is generated by two sources (Lambrechts, 1992) - white noise and 1/f noise. A lower form factor for the electrode (the circumference to surface area ratio) results in a lower white noise level, which implies that the noise generated by circular electrode is lower than that by a band electrode with the same geometric area. The 1/f noise is inversely proportional to the electrode area. Also, magnitude of current output is proportional to the electrode area. This means that current output level and 1/f noise limits the scaling of amperometric sensors to extreme small size for tissue oxygen measurements.

The layout for both configurations were performed using 20, 10, and 5 micron line widths. **Figure 21** is a photograph of the fabricated prototype oxygen sensor **200** (concentric configuration). All noble metal electrodes were made of gold, the material that has been shown to possess the best stability when used as an oxygen catalyzer (Hoare, 1984).

Turning now to the function of each concentric circle shown in **Figure 21**, the middle electrode serves as a working electrode ("WE") **225** at which dissolved oxygen molecules are electrochemically reduced. The GE **227** is wrapped around the

working electrode; this configuration will establish oxygen-saturated or hydrogen-saturated microenvironments during self-calibration cycles. Proceeding from inside to outside, the next concentric circle is used as the reference electrode ("RE") **229**. The outermost electrode in **Figure 21** is the counter electrode ("CE") **231** of this three-electrode cell. It is placed as far as possible from the WE **225** to eliminate electrochemical interference at the WE of byproducts generated at the CE **231**. The GE' **227'** (not shown) is also located remotely from the WE **225** for this same reason.

In the past, pH sensors have also been fabricated on flexible substrates (Cosfret, 1995). **Figure 10A** illustrates a pH sensor structure containing a p-HEMA central dome over a Ag/AgCl electrode. The final fabrication step is the deposition of the outer polymeric membrane containing the pH ionophore. These sensors have performed accurately in preliminary tests *in vivo* in blood for up to two months. The size of these potentiometric sensors are preferably minimized to improve their capability for resolving spatial gradients. Further size reduction of the pH sensors shown in **Figure 10A** may be limited by the manual deposition of the polymeric membrane solution, weaker adhesion to the substrate and high impedance, as the membrane contact area is diminished. Another drawback imposed by the use of polymeric membranes is the potential for leakage and degradation of membrane's plasticizer and ionophore for long-term operation. More recently, work has been done to miniaturize pH sensors by replacing the polymeric membrane by a solid state analogue. The best alternative identified to date is iridium oxide which has been shown to possess excellent pH-sensing capability and can be deposited on the sensor surface using a simple electroplating method (Marzouk, 1998). This new structure is shown in **Figure 10B**.

Self-calibrating O_2 sensors, such as shown in **Figure 21**, have been fabricated by facilities in BMMSL (Biomedical Microsensors laboratory) at North Carolina State University. Tables 2 and 3 summarize a preferred fabrication process of oxygen sensors **200** and pH sensors **201**, respectively.

TABLE 2. Oxygen Sensor Process

Process Steps	Process Details
Substrate selection	3-mil Kapton® VN
Cleaning	Organic solvent cleaning and dehydration
Metal Deposition	DC Magnetron sputtering 200 Å Cr followed by 2000 Å Au
Photolithography	Spin coated 1.3 µm Shipley 1813 photoresist,. Contact exposure with Tamarack Alignment and Exposure System. (Exposure energy optimized for 5-µm linewidth.)
Metal Etching	Wet chemical etching
Cleaning	Organic solvent cleaning and dehydration

Polyimide process	Spin coated 2-μm Pyralin PI-2721 photosensitive polyimide. Contact exposure with Tamarack system. Spin development and thermal curing in atmosphere

TABLE 3. pH Sensor Process

Process Steps	Process Details
Substrate selection	5-mil Kapton® VN
Cleaning	Organic solvent cleaning and dehydration
Metal Deposition	DC Magnetron sputtering 200 Å Ti followed by 2000 Å Pt with shadowmask
Cleaning	Organic solvent clean and dehydration
Polyimide process	Spin coated 5-μm Pyralin PI-2721 photosensitive polyimide. Contact exposure with Tamarack system. Spin development and thermal curing in atmosphere
Electrodeposition	Electroplate IrO_x according to the established method (Marzouk, 1998)

Another preferred embodiment of an *in situ* sensor unit is shown in **Figure 22** as a combination pH/O_2 sensor unit **300**. As the combination sensor unit **300** assumes smaller feature sizes, the area of the generating electrode and, thus, its current carrying capacity, is reduced. A smaller structure will also enable the new sensors to be employed in linear arrays for gradient measurements. The microenvironment of the smaller sensor may require less oxygen to become saturated. Once the GE **327** has established a saturated microenvironment, these conditions will be dissipated rapidly unless structural measures are taken to delay oxygen and pH equilibration. Hence, the self-calibrating design can employ a recessed structure (a micropool) to sustain the saturated microenvironment for a limited sensor calibration period. Thus, a 3-dimensional micropool can be configured by using layers of photosensitive polymers to build walls to confine the working and generating electrodes **325, 327**. The volume of the micropool can also determine the overall sensor unit **300** performance and the time period needed for calibration. A near optimum design can be determined by iterating several of the design parameters in various fabrication runs. It is noted that some surface degradation and adhesion problems at the polyimide/metal interface at the electrode edges were observed during prototype experiments (at current densities exceeding $10mA/cm^2$).

The conventional Clark oxygen sensor contains a reference electrode (anode) and a working electrode (cathode) located in the same compartment encapsulated by hydrophobic, electrically non-conducting membrane. In contrast, the instant design separates the RE **329** and WE **325** to allow a space for the GE **227** (positioned therebetween and placed to control the micro environment of the WE **225**) as

illustrated in **Figures 21** and **22**. This new arrangement is in contrast to the conventional Clark sensor, which may not be suitable for long-term implantation due to the risk of membrane rupture and the subsequent degradation of the sensor's internal filling solution. In this design, the separated RE and WE are electrically coupled via a hydrophilic permeable membrane and tissue fluids. This separated configuration for the RE and WE can cause difficulties due to increased solution resistance when the anode is very far from the cathode. However, the 3-electrode system reduces this effect. Another difficulty can be introduced by WE surface contamination due to direct contact with components of tissue fluid that penetrate the permeable membrane. As such, it is preferred that the electrode material used be selected to reduce this behavior. For example, it has demonstrated (Holmstrom, 1998) that a bare gold electrode, implanted up to 4 years for oxygen monitoring, absorbed less blood proteins than a glassy carbon electrode, and no adverse tissue reactions were observed.

To minimize any electrostatic coupling between the 3-electrode cell and generating current source, the operation of the sensor **300** is preferably divided into separate calibration and measurement modes. To simplify the device structure, the counter electrode (CE) will preferably serve a dual as the counter-generating electrode (GE') of generating source. Thus, a single electrode that can be switched between the two operational modes and can serve both functions.

Preferably, to reduce the feature size and reliably form same during fabrication, a silicon wafer-supported flexible substrate process is used to reduce thermal expansions and surface roughness distortions. In this fabrication process, polyimide (DuPont P12723) is spin-cast to a thickness of about 25 μm onto a thermal oxide coated silicon wafer. After all sensor processing steps have been completed, the wafer is soaked in a dilute H.F. solution. The thermal oxide is etched away and thereby releasing the flexible polyimide substrate and its sensor structures.

A recessed sensor structure can also be implemented using photosensitive polymer materials. Thicknesses of up to 30 μm can be obtained with a 2-step spin-coating procedure. Other materials and configurations are also available for this purpose. For example, a dry film (DuPont Pyralux or Vacrel which have thicknesses of 25 to 100 μm) can be laminated over the device using a thermal vacuum process. The highest aspect ratio (depth:width) for the micropool that can be fabricated using these laminated films is typically about 1:1. This ratio can be maintained for depths from 10 to 100 μm.

Platinum is known as the best noble metal electrode for water electrolysis and is easily deposited and patterned using microfabrication technology. In previous experiments with physiological solutions containing rich chloride ions, surface

chloridation of gold generating electrodes was observed during the positive potential region of water electrolysis. This problem should be alleviated by replacing the gold generating and counter electrodes with platinum. For simplicity, in photo-processing steps, a titanium platinum layer will serve as both electrodes and wiring leads. To generate the other electrode surfaces, gold can be electroplated (for the working electrode) and silver (for the reference electrode) onto the platinum layer. For the pH sensor, iridium oxide will also be plated. The devices are designed so that the electroplating steps are self-aligning, and no additional photopatterning will be required. These procedures have already been established (Marzouk, 1998). Currently, the preferred permeable membrane material is p-HEMA covered with polystyrene or collodion (Kreuzer, 1980).

The overall process sequence is shown in **Figures 23A-23C**. Platinum is deposited by sputtering and then patterned by photolithography. Next, a thin layer of polyimide is spin-coated and patterned to define the various electrode areas and to insulate the wiring conductors. Then a thick polymer micropool is defined around working electrode and reference electrode area by a lamination process. Next, gold (as the oxygen catalyzer) or iridium oxide (as the pH-sensitive layer) will be electroplated, followed the plating and chloridation of silver (as the RE). Finally, a permeable membrane is cast by micromanipulation and cured. In operation, it should be noted that with continuous polarizing voltage during oxygen sensor operation, one disadvantage can be a relatively large oxygen consumption and power consumption as well as aging effect. This power consumption is preferably reduced to provide electrode stability. Thus, intermittent or periodic measurement are preferably instituted with a potential step. Necessary calibration parameters such as current density and duration can be determined for proper calibration of periodic measurements.

The present invention is explained further in the following examples. These examples are for illustrative purposes only, and are not to be construed as limiting of the invention.

EXAMPLE

A patient presents with an unresectable lung cancer (adenocarcinoma or squamous cell). The conventional accepted treatment is a combination of radiation and chemotherapy. The radiation is given everyday, Monday through Friday, and the chemotherapy (taxol and cisplatin) are administered either once a week in low doses or every three weeks in higher doses. All patients are treated in substantially the same manner and the expected response rate is between 50-75%. Therapy is not individualized despite the fact that it is known that oxygen levels, pH, and particularly, cell doubling times, may vary widely between patients.

The availability of the methods, systems, and implantable sensors of the present invention which are configured to monitor pH, oxygen, and radiation, now offer a more customized approach to therapy. The sensors can be positioned in situ in the tumor at different penetration depths or across different regions of the tumor to provide regional specific information. Specific values or oxygen, pH, and cell proliferation can be established either prior to initiation of treatment by a predictive statistical norm in an established data base, or during initial treatment to define relative values, the specific values are identified as either a "go" for treatment or a "no go" for treatment to determine when and if a treatment should be commenced. A monitoring algorithm can be used to quantify important values of variables and an affirmative attempt can be made to correct each variable to reach or approximate the desired specific levels at treatment. For example, to manipulate the tumor to achieve oxygenation of about 50-52mm Hg over a substantial volume of the tumor, as well as to exhibit a lower tumor pH of about 6.8, and to stimulate or identify and deliver during periods of increased cell proliferation.

Following the initial dose of radiation or chemotherapy, each variable will be monitored to determine an appropriate time (associated with a favorable treatment period) to deliver the next dose of radiation and/or chemotherapy. Preferably, each patient is monitored at least four times each day following treatment to establish a specific response pattern for an individual patient. Utilizing this ongoing, periodic monitoring approach can allow delivery of any cytotoxic agent in a more precise and favorable manner and/or to withhold treatment during tumor treatment resistant periods. It is preferably to treat when all variables indicate that the tumor is vulnerable such as when there is an indication of high oxygenation level, low pH, and increased cell proliferation. It the variables do not synchronize to indicate a favorable index at the same time, then a statistical regression analysis can be identified to define an appropriate treatment time. It will be appreciated that in addition to radiation and chemotherapy, hyperthermia and/or other treatments can be incorporated into the treatment protocol, especially in tumors exhibiting a high hypoxic fraction. This can allow for increased cell kill, after which the kinetics of the tumor will change and allow for more precise delivery of the radiation and/or chemotherapy. Thus, the individualized treatment will allow the delivery of cytotoxic agents at a favorable treatment time to achieve increased tumor cell kill, and thereby increase the response of the tumor to the treatment. In this example, when a satisfactory response has been obtained, the tumor can be removed.

In summary, the individualization of therapy can now be instituted based on obtaining information on the dynamic changes within each individual patient's tumor. This information should lead to increase tumor cell kill, increased survival and

decreased morbidity. This should translate into a decrease in the cost of treating patients by a decrease in morbidity and therefore less hospitalization; increase the effectiveness of cytotoxic agents by allowing for delivery of increased dose or even a decrease in the dose through more efficient timing of delivery of the cytotoxic. The present invention can monitor and provide information on dynamic changes occurring within a tumor.

Calibration of in vivo oxygen and pH sensor systems according to the present invention will now be discussed in greater detail. It will be understood that the term "tissue," as used herein, can include organs and other biological matter in a biological body. For example, the term tissue be used to describe organs or other biological matter in a human body. The term "environment" can describe mediums such as water, blood, tissue or other matter that is found in a biological medium, such as a body.

As will be appreciated by one of skill in the art, the present invention may be embodied as methods or devices. Accordingly, the present invention may take the form of a hardware embodiment, a software embodiment or an embodiment combining software and hardware aspects.

The present invention is also described using flowchart illustrations and block diagrams. It will be understood that each block (of the flowchart illustrations and block diagrams), and combinations of blocks, can be implemented by computer program instructions. These program instructions may be provided to a processor(s) within the sensor assemblies such that the instructions which execute on the processor(s) create means for implementing the functions specified in the block or blocks. The computer program instructions may be executed by the processor(s) to cause a series of operational steps to be performed by the processor(s) to produce a computer implemented process such that the instructions which execute on the processor(s) provide steps for implementing the functions specified in the block or blocks.

Accordingly, the blocks support combinations of means for performing the specified functions, combinations of steps for performing the specified functions and program instruction means for performing the specified functions. It will also be understood that each block, and combinations of blocks, can be implemented by special purpose hardware-based systems which perform the specified functions or steps, or combinations of special purpose hardware and computer instructions.

Figure 24 is a block diagram of an embodiment of an in vivo oxygen sensor system **2400** according to the present invention. The in vivo oxygen sensor system **2400** can include an in vivo counter electrode **2445** that has a circular shape with an opening therein and is electrically coupled to an in vivo oxygen sensor circuit **2425**.

The circular shape defines an interior region that may be accessed via the opening. An in vivo reference electrode **2450** is in the interior region and is electrically coupled to the in vivo oxygen sensor circuit **2425**. Other shapes and configurations can be used.

First and second in vivo generating electrodes **2405, 2420** are spaced-apart by about 100 micrometers and are electrically coupled to a current source **2415**. In this embodiment, the first in vivo generating electrode **2405** has a open circular shape and is located in the interior region. Other shapes and configurations can be used.

An in vivo working electrode **2410** is located in the interior region and is electrically coupled to the oxygen sensor circuit **2425**. The oxygen sensor circuit **2425** can be used to determine the level of oxygen that is present a region **2401** proximate to the in vivo working electrode **2410**.

During oxygen measurement operations, the in vivo oxygen sensor circuit **2425** can generate an output that indicates the level of a constituent element of an environment proximate to the in vivo working electrode **2410**. In particular, the level of the constituent element can cause a current to flow in the environment that is proportional to the level. For example, more current may flow when the region **2401** is saturated with oxygen than when the region **2401** is depleted of oxygen. The in vivo oxygen sensor **2425** can, therefore, be used to monitor the level of oxygen in a tissue, such as a tumor, over a long period of time as described above. As used herein, the term "constituent element" includes components of an environment in which a process, such as electrolysis is carried out. For example, constituent elements of a water environment can include oxygen molecules, hydrogen molecules, hydrogen ions, and hydroxyl ions. Electrolysis is well understood by those having skill in the art and will not be discussed further herein.

The first and second in vivo generating electrodes **2405, 2420** can be used to control the level of oxygen proximate to the in vivo sensor electrode **2410** so that the in vivo oxygen sensor circuit **2425** can be periodically calibrated. For example, the in vivo oxygen sensor circuit **2425** can be calibrated by first saturating the region **2401** with oxygen and determining a first level of oxygen at the in vivo working electrode **2410**. Next, the region **2401** can be depleted of oxygen and a second level of oxygen can be determined at the in vivo working electrode **2410**.

The first and second levels of oxygen can be used to more accurately determine a third level of the constituent element measured during operation. For example, the in vivo oxygen sensor circuit **2425** can be assumed to have linear operation at levels between the saturated and depleted levels. A more accurate oxygen level can be provided by determining an oxygen level that is in the same

proportion to the sensor output as the sensor outputs associated with the saturated and depleted levels.

In particular, the current source **2415** can generate a current, i, that flows in the environment in either direction depending on the direction of the current generated by the current source **2415**. For example, the current source **2415** can generate a first current **2430** that flows from the first in vivo generating electrode **2405** to the second in vivo generating electrode **2420**. The current source **2415** can generate a second current **2435** that flows from the second in vivo generating electrode **2420** to the first in vivo generating electrode **2405**.

The current flowing to/from the first in vivo generating electrode **2405** can generate a constituent element of the environment in the region **2401** proximate to the in vivo working electrode **2410**. For example, when the first current **2430** flows, oxygen can be generated at the first in vivo generating electrode **2405** to saturate the region **2401** with oxygen. Alternatively, when the second current **2435** flows, hydrogen can be generated at the first in vivo generating electrode **2405**, thereby depleting the region **2401** of oxygen. Therefore, the region **2401** can have first or second levels of the constituent element depending on the direction of the current flow.

An in vivo oxygen sensor circuit **2425** according to the present invention can be a Clark type sensor such as those shown in **Figures 25A** and **25B**. As shown in **Figure 25A**, a two electrode type Clark sensor can include first and second amplifiers **2501**, **2502** electrically coupled through an environment **2505**. In operation, the level of oxygen in environment **2505** can cause a proportional current, i, to flow from a reference electrode **2510** coupled to the first amplifier **2501** through the environment **2505** to a working electrode **2525** coupled to the second amplifier **2502**.

According to **Figure 25B**, a three electrode type Clark sensor can provide improved operation over the two electrode type Clark sensor shown in **Figure 25A**. In particular, the 3 electrode type Clark sensor can include a counter electrode **2515** coupled to the output of the first amplifier **2501** and the reference electrode **2510** coupled to an input of the first amplifier **2501**. A current, i, flows from the counter electrode **2515** to the working electrode **2525**. The current can be proportional to the level of oxygen that is present in the environment **2505**.

It will be understood that the present invention may also be utilized to calibrate other types of sensor circuits. For example, the present invention may also be utilized to calibrate in vivo glucose sensor circuits. In particular, the working electrode **2525** can include a glucose selective enzyme, such as a Glucose OxiDase (GOD), layer thereon. During operation, as one glucose molecule is detected (consumed) by the GOD, one oxygen molecule can also be consumed to complete

GOD reaction (*i.e.*, oxygen is a cofactor of the reaction). The presence of glucose can be detected by a decrease in oxygen sensor circuit output as compared to a normal oxygen output. A high glucose level can cause a low oxygen level, and a low glucose level can cause a high oxygen level. Accordingly, a high glucose level may result in a low output current in a glucose sensor circuit and a low glucose level may result in high current in the glucose sensor circuit. Glucose sensor circuits are described further, for example, in US Patent 5,791,344 entitled "Patient Monitoring System" to Schulman, et al.

It will also be understood that methods and electrode assemblies according to the present invention can be embodied in probes that are inserted in vivo near a tissue to be monitored. For example, probes according to the present invention can be inserted into a body proximate to a tissue to be monitored and calibrated therein. The probe can thereby provide more accurate oxygen, pH or other data. After the data is provided, the probe may be withdrawn. Probes are further discussed, for example, in "In Vivo Evaluation of Monopolar Intravascular pO_2 Electrodes," Pediatric Research, Vol. 13, 1979, pp. 821-826, by Beran et al.

Figure 26 is an enlarged illustration of an embodiment of an oxygen electrode assembly **2600** according to the present invention. In particular, the oxygen electrode assembly **2600** can be used to calibrate an in vivo oxygen sensor circuit. The oxygen electrode assembly **2600** can be on a flexible substrate **2601** which can be Kapton® or another material that is suitable for tissue-implantation and can be flexed to allow the electrode assembly **2600** to conform to a surface of biological matter. A counter electrode **2640** on the substrate **2601** can have a circular shape with a first opening therein. The circular shape defines an interior region with access thereto via the first opening. The counter electrode **2640** is electrically coupled to the sensor circuit by a conductor **2655** which can be a metal such as gold or platinum. Other materials and configurations and configurations may be used.

A reference electrode **2630** is located in the interior region adjacent to the counter electrode **2640**. The reference electrode **2630** can have a circular shape with a second opening therein. The reference electrode **2630** is electrically coupled to the sensor by a conductor **2650** that passes through the first opening. The spacing between the reference electrode **2630** and the counter electrode **2630** is preferably about 1 micrometer. The reference electrode **2630** can be a silver/silver chloride composition. Other materials and configurations may be used.

A generating electrode **2620** is located in the interior region adjacent to the reference electrode **2630**. The generating electrode **2620** can have a circular shape with a third opening therein. The generating electrode **2620** is electrically coupled to a current source (not shown) by a conductor **2665** that passes through the first and

second openings. The spacing between the generating electrode **2620** and the reference electrode **2630** is preferably about 1 micrometer. The generating electrode **2620** can be a metal such as gold or platinum. Other materials and configurations may be used.

A working electrode **2605** is located in the interior region adjacent to the generating electrode **2620**. The working electrode **2605** can have a circular shape as shown or other shapes may be used. The working electrode **2605** is electrically coupled to the sensor (not shown) by a conductor **2660** that passes through the first, second, and third openings. The spacing between the working electrode **2605** and the generating electrode **2620** is preferably about 1 micrometer. The working electrode **2605** can be a metal such as gold or platinum. Other materials and configurations may be used.

Locating the generating electrode **2620** close to the working electrode **2605** may increase the likelihood that level of oxygen generated will be delivered proximate to working electrode **2605**. Moreover, the circular shape of the generating electrode **2620** may provide a more uniform distribution of oxygen in the saturated and depleted modes proximate to the working electrode **2605**. Although **Figure 26** shows the electrodes as electrically coupled to the sensor or current source via conductors on the substrate **2601**, it will be understood that other routing paths may be used. For example, the substrate can be a multi-layered substrate on which the conductors can be formed, thereby reducing conductor routing problems.

Figure 27 is an enlarged illustration of an embodiment of an oxygen electrode assembly **2700** according to the present invention. In particular, the oxygen electrode assembly **2700** can be used to calibrate an in vivo oxygen sensor circuit. The oxygen electrode assembly **2700** can be on a flexible substrate **2701** which can be Kapton® or another material that is suitable for tissue-implantation and can be flexed to allow the oxygen electrode assembly **2700** to conform to a surface of biological matter. Counter electrodes **2740a, 2740b** on the substrate **2701** can be strip shaped conductors that are electrically coupled together and define an interior region therebetween. The counter electrodes **2740a, 2740b** are electrically coupled to the in vivo sensor by a conductor **2720** which can be a metal such as gold or platinum. Other materials and configurations may be used.

Reference electrodes **2735a, 2735b** are located in the interior region adjacent to the counter electrodes **2740a, 2740b**. The reference electrodes **2735a, 2735b** can be strip shaped conductors that are electrically coupled together. The reference electrodes **2735a, 2735b** are electrically coupled to the in vivo sensor by a conductor **2715**. The spacing between the reference electrode **2735a, 2735b** and the counter electrodes **2740a, 2740b** is preferably about 1 micrometer respectively. The reference

electrodes **2735a, 2735b** can be a silver/silver chloride composition. Other materials and configurations may be used.

Generating electrodes **2730a, 2730b** are located in the interior region adjacent to the reference electrodes **2735a, 2735b** respectively. The generating electrodes **2730a, 2730b** can be strip shaped conductors that are electrically coupled together. The generating electrodes **2730a, 2730b** are electrically coupled to a current source (not shown) by a conductor **2710**. The spacing between the generating electrodes **2730a, 2730b** and the reference electrodes **2735a, 2735b** is preferably about 1 micrometer respectively. The generating electrodes **2730a, 2730b** can be a metal such as gold or platinum. Other materials and configurations may be used.

A working electrode **2725** is located in the interior region adjacent to the generating electrodes **2730a, 2730b**. In one embodiment, the working electrode **2725** is located at a center of the interior region. The working electrode **2725** can be a strip shaped conductor that is electrically coupled to the in vivo sensor (not shown) by a conductor **2705**. The spacing between the working electrode **2725** and the generating electrodes **2730a, 2730b** is preferably about 1 micrometer. The working electrode **2725** can be a metal such as gold or platinum. Other materials and configurations may be used.

Although **Figure 27** shows the electrodes as electrically coupled to the sensor or current source via conductors on the substrate **2701**, it will be understood that other routing paths may be used. For example, the substrate can be a multi-layered substrate on which the conductors can be formed, thereby reducing conductor routing problems.

Figure 29 is an enlarged cross-sectional view of an embodiment of a portion of an electrode assembly **2900** according to the present invention. In particular, the electrode assembly **2900** can represent any of the electrodes described here. The electrode assembly **2900** can be on a flexible substrate **2901** which can be Kapton® or another material that is suitable for tissue-implantation and can be flexed to allow the electrode assembly **2900** to conform to a surface of biological matter.

An electrode **2910** is on the substrate **2901** and can be any portion of any of the electrodes described herein. An insulating layer **2905** on the electrode **2910** includes a hole **2915** which exposes a portion of the electrode **2910**. The insulating layer **2905** can overlap the electrode **2910** on each side thereof. In particular, a dimension, A, of the electrode **2910** can be covered by the insulating layer **2905** on either side of the hole **2915**. The exposed portion of the electrode **2910** has a width, B, which is about equal to the widths A covered by the insulating layer **2905** on either side of the hole **2915**. In a preferred embodiment, the dimensions A and B are about 1 micrometer.

The length, L, of the electrode described above can be about 50 times the width of the exposed portion of the electrode. For example, in the one embodiment of the present invention, the exposed width of the electrode is about 1 micrometer and the length is about 50 micrometers.

Figure 28 is an enlarged illustration of another embodiment of an oxygen electrode assembly **2800** according to the present invention. In particular, the oxygen electrode assembly **2800** can be used to calibrate an in vivo oxygen sensor circuit. The oxygen electrode assembly **2800** can be on a flexible substrate **2801** which can be Kapton® or another material that is suitable for tissue-implantation and can be flexed to allow the oxygen electrode assembly **2800** to conform to a surface of biological matter.

As shown in **Figure 28**, a counter electrode **2810** on the substrate **2801** can have a circular shape with a first opening therein. The circular shape defines an interior region with access thereto via the first opening. The counter electrode **2810** is electrically coupled to the in vivo sensor by a conductor **2830** which can be a metal such as gold or platinum. Other materials and configurations may be used.

A generating electrode **2815** is located in the interior region adjacent to the counter electrode **2810**. The generating electrode **2815** can have a circular shape with a second opening therein. The generating electrode **2815** is electrically coupled to a current source (not shown) by a conductor **2845** that passes through the first opening. The spacing between the generating electrode **2815** and the counter electrode **2810** is preferably about 1 micrometer. The generating electrode **2815** can be a metal such as gold or platinum. Other materials and configurations may be used.

A reference electrode **2825** is located in the second opening in the interior region adjacent to the generating electrode **2815**. The reference electrode **2825** can be a tab shape conductor. In particular, the second opening can be larger than the third in **Figure 26**, thereby allowing the reference electrode **2825** to be located closer to a working electrode **2820**. The reference electrode **2825** is electrically coupled to the in vivo sensor by a conductor **2835** that passes through the first opening. The spacing between the reference electrode **2825** and the counter electrode **2810** is preferably about 1 micrometer. The spacing between the reference electrode **2825** and the generating electrode **2815** can be about 1 micrometer. The reference electrode **2825** can be a silver/silver chloride composition. Other materials and configurations may be used.

The working electrode **2820** is located in the interior region adjacent to the generating electrode **2815** and the reference electrode **2825**. The working electrode **2820** can have a circular shape as shown or other shapes may be used. In one embodiment, the working electrode **2820** is located in the center of the interior region.

The working electrode **2820** is electrically coupled to the in vivo sensor (not shown) by a conductor **2840** that passes through the first and second openings. The spacing between the working electrode **2820** and the generating and reference electrodes **2815**, **2825** is preferably about 1 micrometer. The working electrode **2820** can be a metal such as gold or platinum. Other materials and configurations may be used.

In comparison, for example, to the embodiment of **Figure 27**, locating the reference electrode **2825** closer to the working electrode **2820** may provide improved sensor performance. Moreover, maintaining the circular shape of the generating electrode **2815** and locating the reference electrode **2825** close to the working electrode **2820**, may provide a more uniform distribution of oxygen and increase the likelihood that level of oxygen generated will be delivered proximate to working electrode **2820**.

Although **Figure 28** shows the electrodes as electrically coupled to the sensor or current source via conductors on the substrate **2801**, it will be understood that other routing paths may be used. For example, the substrate can be a multi-layered substrate on which the conductors can be formed, thereby reducing conductor routing problems.

Figure 30 is a flowchart that illustrates operations of embodiments of oxygen electrode assemblies according to the present invention. In particular, an oxygen sensor circuit can be calibrated by saturating the environment proximate to the in vivo working electrode with oxygen (block **3001**). In one embodiment, current is provided to the in vivo generating electrode for approximately 1 minute to produce an oxygen saturated environment proximate to the in vivo working electrode. After the environment is saturated, the generation of oxygen may be discontinued.

A first level of oxygen is then determined using the in vivo sensor circuit (block **3002**). In particular, the amount of current flowing in the in vivo sensor circuit may be used to determine the first level of oxygen proximate to the in vivo working electrode. In one embodiment, the first level of oxygen is determined immediately after the generation of oxygen ceases. The oxygen generated (block **3001**) is then allowed to dissipate. In one embodiment, the oxygen is allowed to dissipate for approximately 1 minute.

After the saturated oxygen environment has dissipated, an oxygen depleted environment is generated at the in vivo generating electrode by generating hydrogen at the in vivo generating electrode (block **3003**). In particular, the direction of the current can be reversed to generate hydrogen at the in vivo generating electrode. In one embodiment, current is provided to the in vivo generating electrode for approximately 1 minute to produce the oxygen depleted environment proximate to the

in vivo working electrode. After the environment is depleted of oxygen, the generation of hydrogen may be discontinued.

A second level of oxygen is then determined using the in vivo sensor circuit (block **3004**). In particular, the amount of current flowing in the in vivo sensor circuit may be used to determine the second level of oxygen proximate to the in vivo working electrode. In one embodiment, the second level of oxygen is determined immediately after the generation of hydrogen ceases.

Figure 31 is a block diagram of an embodiment of an in vivo pH sensor system **3100** according to the present invention. In particular, the in vivo pH sensor system **3100** can be used to calibrate an in vivo pH sensor circuit. The in vivo pH sensor system **3100** can be on a flexible substrate **3150** which can be Kapton® or another material that is suitable for tissue-implantation and can be flexed to allow the in vivo pH sensor system **3100** to conform to a surface of biological matter.

The in vivo pH sensor system **3100** can include an in vivo pH electrode **3110** and an in vivo reference electrode **3130** that are electrically coupled to a pH sensor circuit **3125**, such as a voltmeter. The in vivo pH electrode **3110** can be made of iridium oxide or PolyVinylChloride (PVC) which can generate a voltage related to the pH level of the environment.

A first in vivo generating electrode **3105** and a second in vivo generating electrode **3120** are spaced-apart on the substrate **3150** by about 100 micrometers and are electrically coupled to a current source **3115**. The first in vivo generating electrode **3105** is located proximate to the in vivo pH electrode **3110**.

The pH sensor circuit **3125** can be used to determine a pH level of the environment proximate to the in vivo pH electrode **3110**. As shown in the pH titration curve **3201** shown in **Figure 32**, a level of H+ and OH- ions in the environment is related to the pH of the environment. In particular, if the environment includes more H+ ions than OH- ions, the environment may be relatively acidic. If the environment includes more OH- ions than H+ ions, the environment may be relatively basic. Moreover, the level of H+ and OH- ions in the environment can cause a voltage to develop at the in vivo pH electrode **3110** that is proportional to the pH level. For example, when the level of the OH^- ions in region **3101** increases, the pH can increase (to a pH level 10) which can produce an increased voltage at the in vivo pH electrode **3110**. When level of H+ ions in the region **3101** increase, the pH can decrease (to a pH level 4) which can produce a decreased voltage at the in vivo pH electrode **3110**. The in vivo pH sensor circuit **3125** can, therefore, be used to monitor the pH level near a tissue, such as a tumor, over a long period of time as described above.

The first and second in vivo generating electrodes **3105, 3120** can be used to control the level of H+/OH- ions proximate to the in vivo pH electrode **3110** so that the pH sensor circuit **3125** can be calibrated. For example, the pH sensor circuit **3125** can be calibrated by generating a changing level of H+/OH- ions in the region **3101** which change the voltage at the in vivo pH electrode **3110**. The voltage generated at the in vivo pH electrode **3110** can be monitored to determine a rate of change of the voltage **3210** as the level of H+/OH- ions changes. In one embodiment, the maximum value of the rate of change of the voltage can indicate a neutral pH level 3205 (pH 7) in the environment.

The rate of change of the voltage **3205** can be used to more accurately determine a pH level of the environment during operation. For example, the in vivo pH electrode **3110** can be assumed to generate voltages in a linear range for pH levels between the high and low pH. A more accurate pH level can be provided by determining a pH level that is in the same proportion to an observed voltage as the voltage associated with the rate of change of the voltage **3205**.

In particular, the current source **3115** can generate a current, i, that flows in the environment in either direction depending on the direction of the current generated by the current source **3115**. For example, the current source **3115** can generate a first current **3136** that flows from the first in vivo generating electrode **3105** to the second in vivo generating electrode **3120**. The current source **3115** can generate a second current **3135** that flows from the second in vivo generating electrode **3120** to the first in vivo generating electrode **3105**.

The current flowing to/from the in vivo generating electrode **3105** can generate a changing level of H+/OH- ions in the region **3101**. For example, when the first current **3136** flows, the level of the H+ ions in the region **3101** can be increased, thereby decreasing the pH of the region **3101**. When the second current **3135** flows, the level of OH- ions in the region **3101** can be increased, thereby increasing the pH level of the region **3101**. Therefore, the region **3101** can have an increasing or decreasing pH level depending on the direction of the current flow.

Figure 33 is an enlarged illustration of an embodiment of an in vivo pH electrode assembly **3300** according to the present invention. In particular, the in vivo pH electrode assembly **3300** can be used to calibrate pH levels generated by an in vivo pH electrode **3320**. The in vivo pH electrode assembly **3300** can be on a flexible substrate **3301** which can be Kapton® or another material that is suitable for tissue-implantation and can be flexed to allow the in vivo pH electrode assembly **3300** to conform to a surface of biological matter.

As shown in **Figure 33**, an in vivo reference electrode **3310** on the substrate **3301** can have a circular shape with a first opening therein. The circular shape defines

an interior region with access thereto via the first opening. The in vivo reference electrode **3310** is electrically coupled to the in vivo measuring pH sensor circuit by a conductor **3330** which can be a metal such as gold or platinum. Other materials and configurations may be used.

A generating electrode **3315** is located in the interior region adjacent to the reference electrode **3310**. The generating electrode **3315** can have a circular shape with a second opening therein. The generating electrode **3315** is electrically coupled to a current source (not shown) by a conductor **3345** that passes through the first opening. The spacing between the generating electrode **3315** and the reference electrode **3310** is preferably about 1 micrometer. The reference electrode **3310** can be a silver/silver chloride composition. Other materials and configurations may be used.

A pH electrode **3320** is located in the interior region adjacent to the generating electrode **3315**. In one embodiment, the pH electrode **3320** can be located at a center of the interior region. The pH electrode **3320** can have a circular shape as shown or other shapes may be used. The pH electrode **3320** is electrically coupled to the in vivo pH sensor circuit (not shown) by a conductor **3302** that passes through the first and second openings. The spacing between the pH electrode **3320** and the generating electrode **3315** is preferably about 1 micrometer. The pH electrode **3320** can be made of iridium oxide or PVC. Other materials and configurations may be used.

Although **Figure 33** shows the electrodes as electrically coupled to the pH sensor circuit or current source via conductors on the substrate **3301**, it will be understood that other routing paths may be used. For example, the substrate **3301** can be a multi-layered substrate inn which the conductors can be routed via multiple signal layers, thereby further reducing conductor routing problems.

Figure 34 is an enlarged illustration of another embodiment of an in vivo pH electrode assembly **3400** according to the present invention. In particular, the pH electrode assembly **3400** can be used to calibrate an in vivo pH sensor circuit. The in vivo pH electrode assembly **3400** can be on a flexible substrate **3401** which can be Kapton® or another material that is suitable for tissue-implantation and can be flexed to allow the in vivo pH electrode assembly **3400** to conform to a surface of biological matter.

As shown in **Figure 34**, an in vivo generating electrode **3405** on the substrate **3401** can have a circular shape with an opening therein. The circular shape defines an interior region with access thereto via the opening. The in vivo generating electrode **3405** is electrically coupled to the current source (not shown) by a conductor **3430** which can be a metal such as gold or platinum. Other materials and configurations may be used.

An in vivo reference electrode **3410** is located in the opening to the interior region adjacent to the generating electrode **3405**. The in vivo reference electrode **3410** can be a tab shaped conductor as shown. The in vivo reference electrode **3410** is electrically coupled to the in vivo pH sensor circuit (not shown) by a conductor **3435** that passes through the opening. The spacing between the in vivo reference electrode **3410** and the in vivo generating electrode **3405** is preferably about 1 micrometer. The in vivo reference electrode **3410** can be a silver/silver chloride composition. Other materials and configurations may be used.

The in vivo pH electrode **3415** can be located in the interior region adjacent to the in vivo generating electrode **3405** and the in vivo reference electrode **3410**. The in vivo pH electrode **3415** can have a circular shape as shown or other shapes may be used. The in vivo pH electrode **3415** is electrically coupled to the in vivo pH sensor circuit (not shown) by a conductor **3402** that passes through the opening. The spacing between the in vivo pH electrode **3415** and the in vivo generating and reference electrodes **3405, 3410** is preferably about 1 micrometer. The in vivo pH electrode **3415** can be made of iridium oxide or PVC. Other materials and configurations may be used.

Figure 35 is a flowchart that illustrates operations of embodiments of in vivo pH electrode assemblies according to the present invention. In particular, an in vivo pH sensor circuit can be calibrated by changing the level of H+/OH- ions proximate to the in vivo pH electrode (block **3501**). In one embodiment, a constant current is provided to the in vivo generating electrode to increase the level of OH- proximate to the in vivo pH electrode. Consequently, the voltage level at the in vivo pH electrode may increase. The rate of increase in the voltage can depend on the magnitude of the current.

The rate of change of the voltage at the in vivo pH electrode is determined as the pH level changes (block **3502**). In particular, as the pH level increases above 4, the rate of change may become approximately linear as shown in **Figure 32**. A rate of change of the voltage can be used to select a voltage level to be associated with a pH level. The selected voltage level can be used to more accurately determine the pH level proximate to the in vivo pH electrode (block **3503**). In particular, the rate of change of the voltage can be used to relate voltages at the in vivo pH electrode to pH levels.

In one embodiment according to the present invention, a maximum rate of change of the voltage at the in vivo pH pH electrode is determined. The voltage level associated with the time when the maximum rate of change occurred is associated with a pH level 7. In operation, a voltage at the in vivo pH electrode can be associated with a pH level using the voltage level associated with the pH level 7.

The foregoing is illustrative of the present invention and is not to be construed as limiting thereof. Although a few exemplary embodiments of this invention have been described, those skilled in the art will readily appreciate that many modifications are possible in the exemplary embodiments without materially departing from the novel teachings and advantages of this invention. Accordingly, all such modifications are intended to be included within the scope of this invention as defined in the claims. In the claims, means-plus-function clauses are intended to cover the structures described herein as performing the recited function and not only structural equivalents but also equivalent structures. Therefore, it is to be understood that the foregoing is illustrative of the present invention and is not to be construed as limited to the specific embodiments disclosed, and that modifications to the disclosed embodiments, as well as other embodiments, are intended to be included within the scope of the appended claims. The invention is defined by the following claims, with equivalents of the claims to be included therein.

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What is Claimed:

1. A method for calibrating an in vivo sensor system, the method comprising the steps of:

generating a constituent element of an environment proximate to an in vivo sensor electrode via an in vivo generating electrode; and

determining a level of the constituent element in the tissue via the in vivo sensor electrode.

2. A method according to Claim 1 further comprising the step of:

calibrating the in vivo sensor circuit based on the level of the constituent element using a chronically tissue-implanted device.

3. A method according to Claim 1, wherein the step of generating comprises the steps of:

generating a first constituent element at a first time at the in vivo generating electrode; and

generating a second constituent element at a second time at the in vivo generating electrode.

4. A method according to Claim 3, wherein the step of determining comprises the steps of:

determining a first level of the constituent element between the first time and the second time; and

determining a second level of the constituent element after the second time.

5. A method according to Claim 3, wherein the step of generating the first constituent element comprises the step of generating oxygen, and wherein the step of generating the second constituent element comprises the step of generating hydrogen.

6. A method according to Claim 3, wherein the step of generating the first constituent element comprises the step of generating an oxygen saturated environment proximate to the in vivo sensor electrode, and wherein the step of generating the second constituent element comprises the step of generating a hydrogen saturated environment proximate to the in vivo sensor electrode.

7. A method according to Claim 1, wherein the step of generating comprises the step of generating the constituent element using electrolysis.

8. A method according to Claim 3, wherein the step of generating the second constituent element is preceded by the step of waiting a time interval to allow the generated first constituent element to dissipate.

9. A method according to Claim 8, wherein the time interval comprises about 1 minute.

10. A method according to Claim 3, wherein the step of generating the first constituent element comprises the step of conducting a current from a first in vivo generating electrode to a second in vivo generating electrode through the tissue.

11. A method according to Claim 10, wherein the step of generating the second constituent element comprises the step of conducting the current from the second in vivo generating electrode to the first in vivo generating electrode through the tissue.

12. A method according to Claim 3, wherein the step of calibrating comprises the step of determining a relationship between a current conducted by the in vivo sensor electrode and a third level of the constituent element using the first and second levels of the constituent element.

13. A method according to Claim 1, wherein the step of generating comprises the step of generating a changing H^+/OH^- ion level proximate to the in vivo sensor electrode.

14. A method according to Claim 13, wherein the step of determining comprises the step of determining a rate of change of a voltage generated at the in vivo sensor electrode in response to the changing H^+/OH^- ion level.

15. A method according to Claim 12, wherein the step of calibrating comprises the steps of:

determining a maximum rate of change of the voltage; and

associating the maximum rate of change of the voltage with a voltage level that is associated with a neutral pH level.

16. A method for calibrating an in vivo sensor system, the method comprising the steps of:

generating a first constituent element of an environment proximate to an in vivo sensor electrode at an in vivo generating electrode;

determining a first level of the first constituent element via the in vivo sensor electrode;

generating a second constituent element of the environment proximate to the in vivo sensor electrode at the in vivo generating electrode; and

determining a second level of the second constituent element using an in vivo sensor electrode.

17. A method according to Claim 16 further comprising the step of:

calibrating the in vivo sensor using an in vivo device.

18. A method according to Claim 17, wherein the step of calibrating comprises the step of determining a relationship between a current in the environment and a third level of the first constituent element using the first and second levels of the constituent elements.

19. A method according to Claim 16, wherein the step of generating the first constituent element comprises the step of generating oxygen, and wherein the step of generating the second constituent element comprises the step of generating hydrogen.

20. A method according to Claim 16, wherein the step of generating the first constituent element comprises the step of generating an oxygen saturated environment, and wherein the step of generating the second constituent element comprises the step of generating an oxygen depleted environment.

21. A method according to Claim 16, wherein the step of generating the first constituent element comprises the step of generating the first constituent element using electrolysis.

22. A method according to Claim 16, wherein the step of generating the second constituent element is preceded by the step of waiting a time interval to allow the generated first level of the first constituent element to dissipate.

23. A method according to Claim 22, wherein the time interval comprises about 1 minute.

24. A method according to Claim 16, wherein the step of generating the first constituent element comprises the step of conducting a current from the first in vivo generating electrode to a second in vivo generating electrode.

25. A method according to Claim 24, wherein the step of generating the second constituent element comprises the step of conducting a current from the second in vivo generating electrode to the first in vivo generating electrode.

26. A method for calibrating an in vivo sensor system, the method comprising the steps of:

generating a changing H^+/OH^- ion level proximate to an in vivo sensor electrode;

determining a rate of change of a voltage at the in vivo sensor electrode generated in response to the changing H^+/OH^- ion level; and

determining a rate of change of the voltage associated with the changing H^+/OH^- ion level.

27. A method according to Claim 26 further comprising the step of:

calibrating the in vivo sensor based on the determined rate of change of the voltage using an in vivo device.

28. A method according to Claim 27, wherein the step of calibrating comprises the step of associating the rate of change of the voltage with a voltage level that is associated with a pH level.

29. A method according to Claim 26, wherein the step of generating comprises the steps of:

generating an increase in OH^- ions and a decrease in H^+ ions proximate to the in vivo sensor electrode; and

generating a decrease in OH^- ions and an increase in H^+ ions proximate to the in vivo sensor electrode.

30. A method according to Claim 29, wherein the generating steps are performed more than once.

31. A method according to Claim 27, wherein the step of calibrating comprises the step of associating a maximum rate of change of the voltage with a voltage level that is associated with a neutral pH level.

32. An electrode assembly comprising:

an in vivo substrate;

an in vivo generating electrode, on the substrate, having a first shape that defines an interior region; and

an in vivo sensor electrode, spaced-apart from the in vivo generating electrode on the substrate, having a second shape and positioned in the interior region in a non-contacting relationship with the in vivo generating electrode.

33. An electrode assembly according to Claim 32, wherein the first shape comprises a u shape and wherein the second shape comprises a strip shape.

34. An electrode assembly according to Claim 32, wherein the in vivo generating electrode and the in vivo sensor electrode are spaced-apart by about 1micrometer.

35. An electrode assembly according to Claim 32, wherein the first shape comprises an open circular shape defining a first central axis therethrough and wherein the second shape comprises a circular shape defining a second central axis therethrough, wherein the first and second central axes are co-axial.

36. An electrode assembly according to Claim 32 further comprising:

a reference electrode, on the substrate, having the first shape and that defines a second interior region, wherein the in vivo generating electrode and the in vivo sensor electrode are located in the second interior region in a non-contacting relationship with the in vivo reference electrode.

37. An electrode assembly according to Claim 36 further comprising:

a counter electrode, on the substrate, having the first shape and that defines a third interior region, wherein the in vivo generating electrode, the in vivo sensor electrode, and the in vivo reference electrode are located in the third interior region in a non-contacting relationship with the in vivo counter electrode.

38. An electrode assembly according to Claim 32 further comprising:

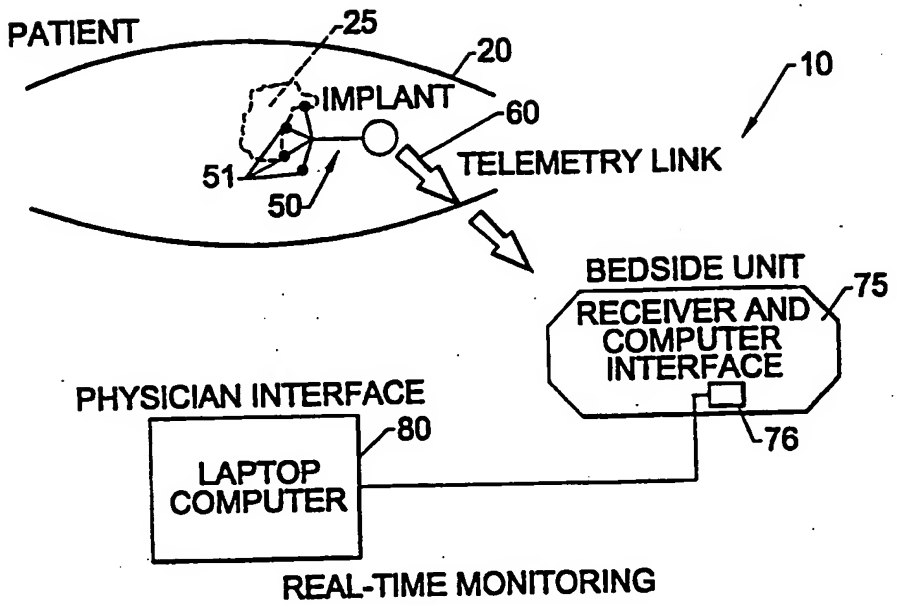
an insulating layer on the in vivo generating electrode and the in vivo sensor electrode, wherein a first portion of the in vivo sensor electrode is exposed through the insulating layer and has the first shape, and wherein a second portion of the in

vivo generating electrode is exposed through the insulating layer and has the second shape.

39. An electrode assembly according to Claim 37, wherein a length of the first portion of the in vivo generating electrode is about 50 times greater than a width of the first portion.

40. An electrode assembly according to Claim 32, wherein the in vivo generating electrode is configured to generate a first constituent element at a first time at the in vivo generating electrode and to generate a second constituent element at a second time at the in vivo generating electrode.

41. An electrode assembly according to Claim 32 further comprising a glucose selective enzyme on the in vivo sensor electrode.


PATIENT
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BEDSIDE UNIT
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PHYSICIAN INTERFACE
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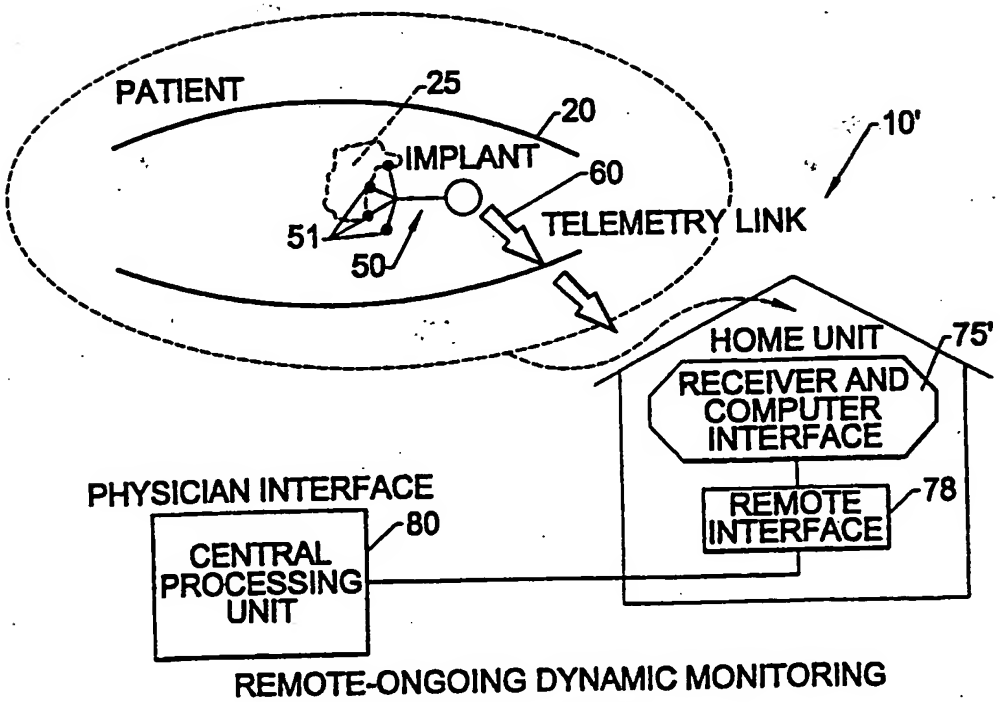
LAPTOP COMPUTER
REAL-TIME MONITORING

FIG. 1A.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RECEIVER AND COMPUTER INTERFACE
REMOTE INTERFACE
78
PHYSICIAN INTERFACE
80
CENTRAL PROCESSING UNIT
REMOTE-ONGOING DYNAMIC MONITORING

FIG. 1B.

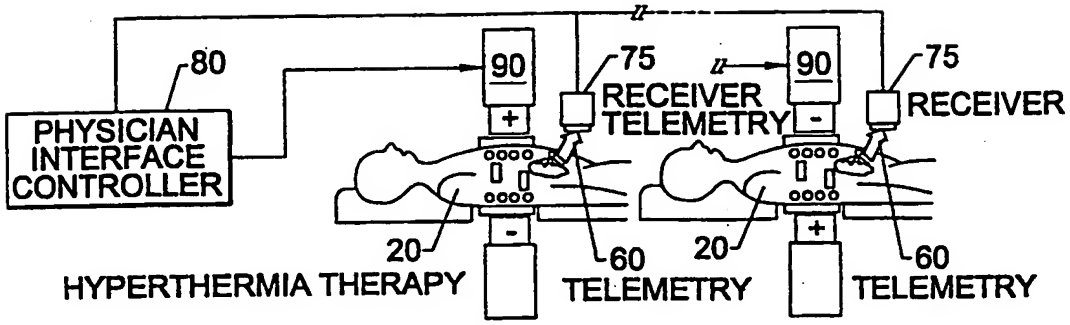

80
PHYSICIAN INTERFACE CONTROLLER
90
+
75
RECEIVER TELEMETRY
90
-
75
RECEIVER
20
HYPERTHERMIA THERAPY
-
60
TELEMETRY
20
+
60
TELEMETRY

FIG. 2A.

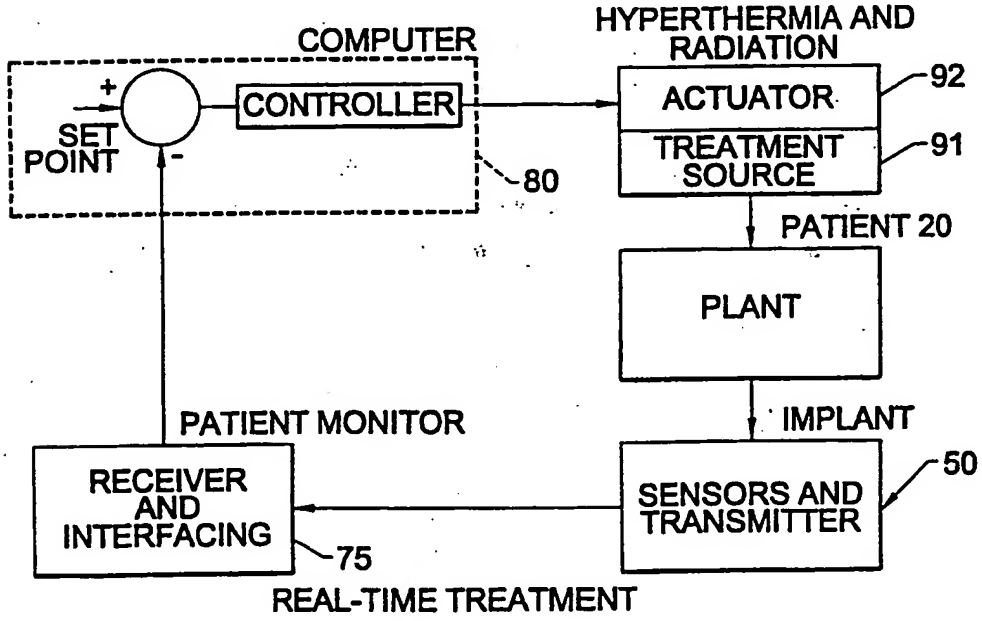

COMPUTER
+
SET POINT
-
CONTROLLER
80
HYPERTHERMIA AND RADIATION
ACTUATOR
92
TREATMENT SOURCE
91
PATIENT 20
PLANT
IMPLANT
PATIENT MONITOR
RECEIVER AND INTERFACING
75
SENSORS AND TRANSMITTER
50
REAL-TIME TREATMENT

FIG. 2B.

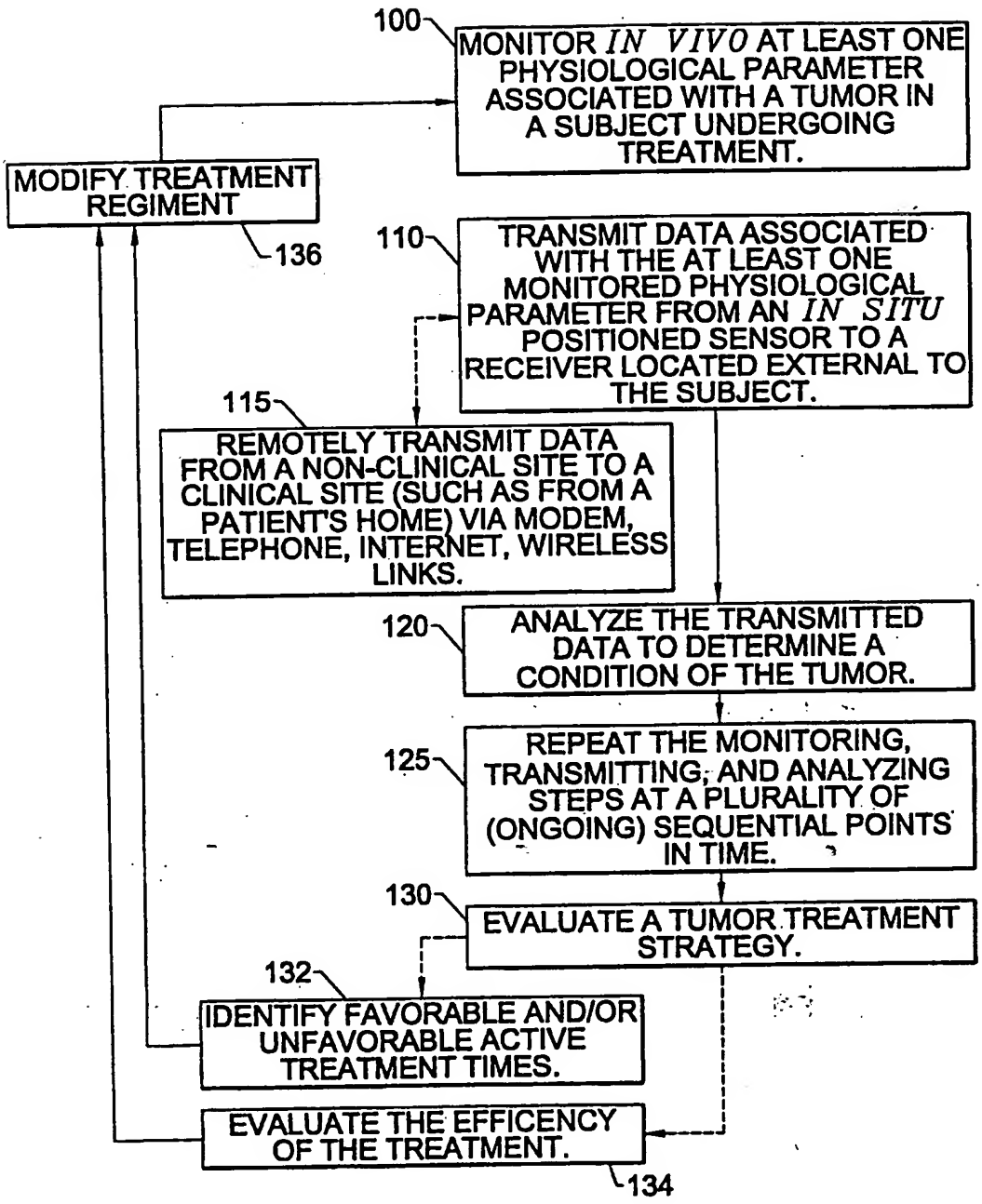

100
MONITOR *IN VIVO* AT LEAST ONE PHYSIOLOGICAL PARAMETER ASSOCIATED WITH A TUMOR IN A SUBJECT UNDERGOING TREATMENT.
MODIFY TREATMENT REGIMENT
136
110
TRANSMIT DATA ASSOCIATED WITH THE AT LEAST ONE MONITORED PHYSIOLOGICAL PARAMETER FROM AN *IN SITU* POSITIONED SENSOR TO A RECEIVER LOCATED EXTERNAL TO THE SUBJECT.
115
REMOTELY TRANSMIT DATA FROM A NON-CLINICAL SITE TO A CLINICAL SITE (SUCH AS FROM A PATIENT'S HOME) VIA MODEM, TELEPHONE, INTERNET, WIRELESS LINKS.
120
ANALYZE THE TRANSMITTED DATA TO DETERMINE A CONDITION OF THE TUMOR.
125
REPEAT THE MONITORING, TRANSMITTING, AND ANALYZING STEPS AT A PLURALITY OF (ONGOING) SEQUENTIAL POINTS IN TIME.
130
EVALUATE A TUMOR TREATMENT STRATEGY.
132
IDENTIFY FAVORABLE AND/OR UNFAVORABLE ACTIVE TREATMENT TIMES.
EVALUATE THE EFFICENCY OF THE TREATMENT.
134

FIG. 3.

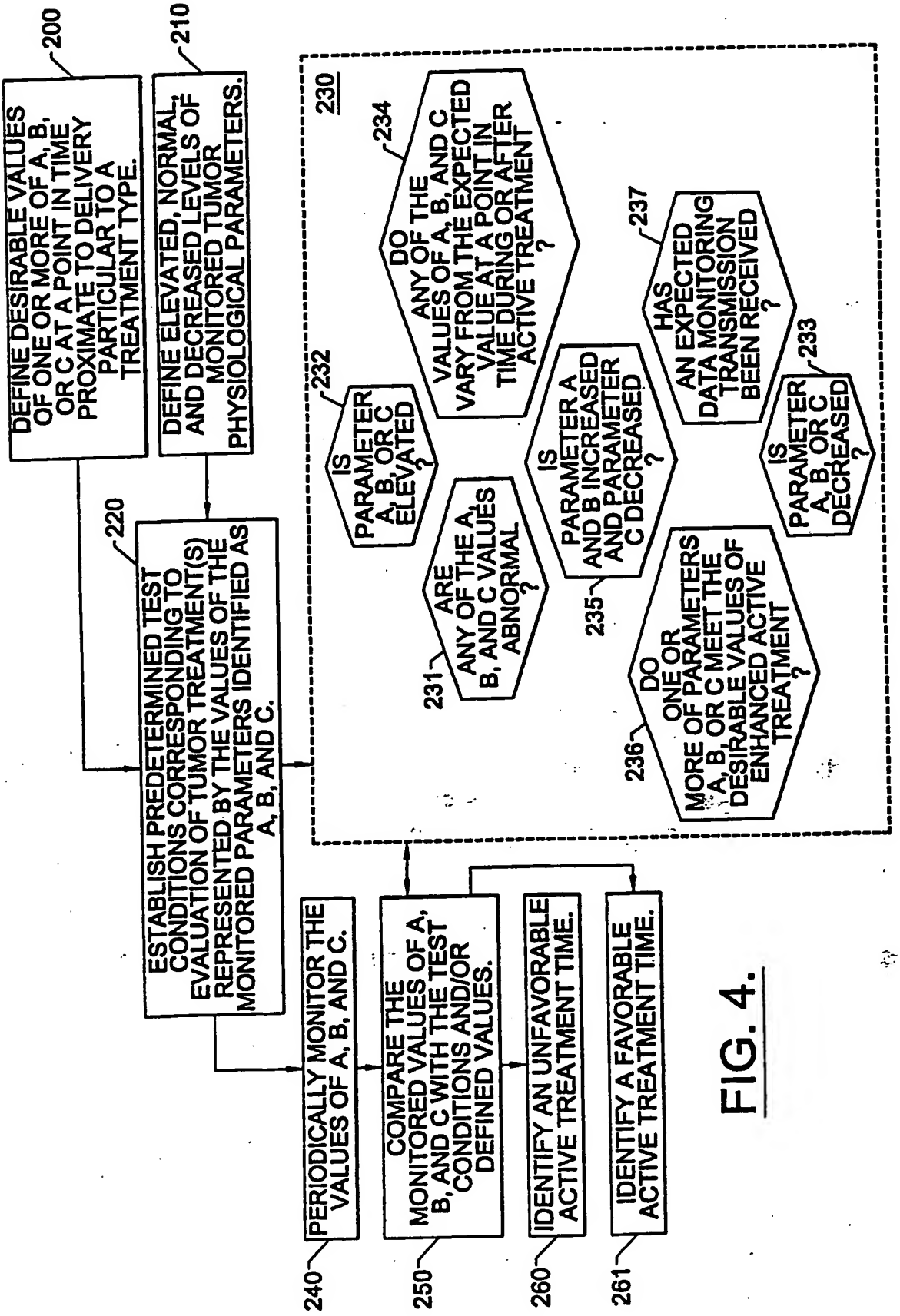

200
DEFINE DESIRABLE VALUES OF ONE OR MORE OF A, B, OR C AT A POINT IN TIME PROXIMATE TO DELIVERY PARTICULAR TO A TREATMENT TYPE.
210
DEFINE ELEVATED, NORMAL, AND DECREASED LEVELS OF MONITORED TUMOR PHYSIOLOGICAL PARAMETERS.
220
ESTABLISH PREDETERMINED TEST CONDITIONS CORRESPONDING TO EVALUATION OF TUMOR TREATMENT(S) REPRESENTED BY THE VALUES OF THE MONITORED PARAMETERS IDENTIFIED AS A, B, AND C.
230
232
IS PARAMETER A, B, OR C ELEVATED ?
234
DO ANY OF THE VALUES OF A, B, AND C VARY FROM THE EXPECTED VALUE AT A POINT IN TIME DURING OR AFTER ACTIVE TREATMENT ?
231
ARE ANY OF THE A, B, AND C VALUES ABNORMAL ?
235
IS PARAMETER A AND B INCREASED AND PARAMETER C DECREASED ?
236
DO ONE OR MORE OF PARAMETERS A, B, OR C MEET THE DESIRABLE VALUES OF ENHANCED ACTIVE TREATMENT ?
237
HAS AN EXPECTED DATA MONITORING TRANSMISSION BEEN RECEIVED ?
233
IS PARAMETER A, B, OR C DECREASED ?
240
PERIODICALLY MONITOR THE VALUES OF A, B, AND C.
250
COMPARE THE MONITORED VALUES OF A, B, AND C WITH THE TEST CONDITIONS AND/OR DEFINED VALUES.
260
IDENTIFY AN UNFAVORABLE ACTIVE TREATMENT TIME.
261
IDENTIFY A FAVORABLE ACTIVE TREATMENT TIME.

FIG. 4.

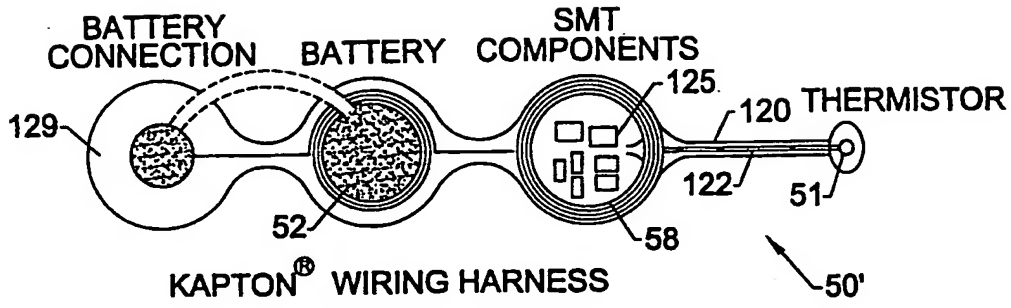


FIG. 5.

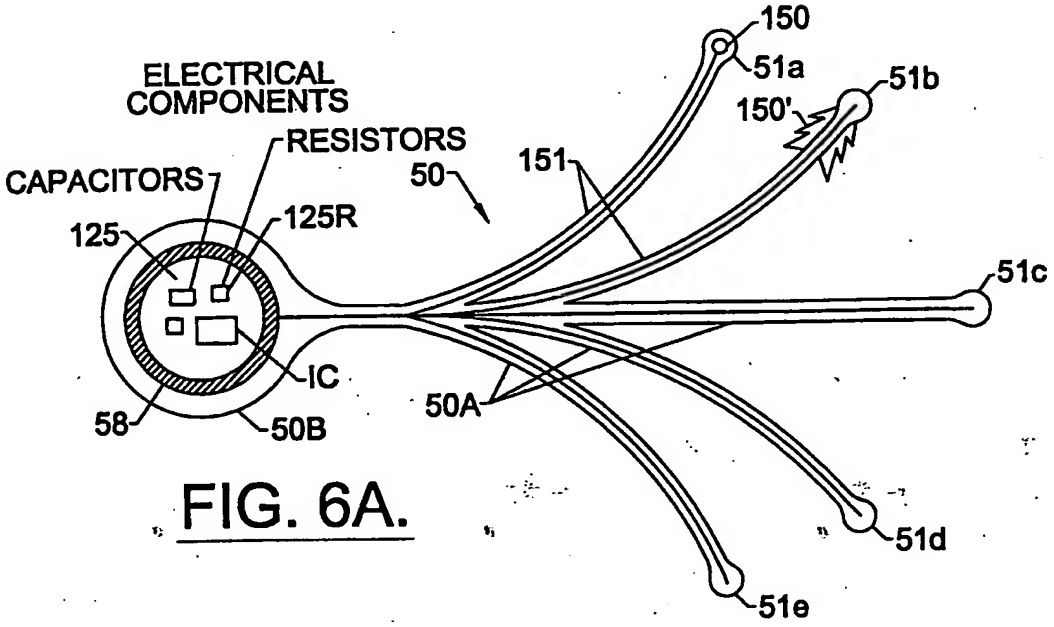


FIG. 6A.

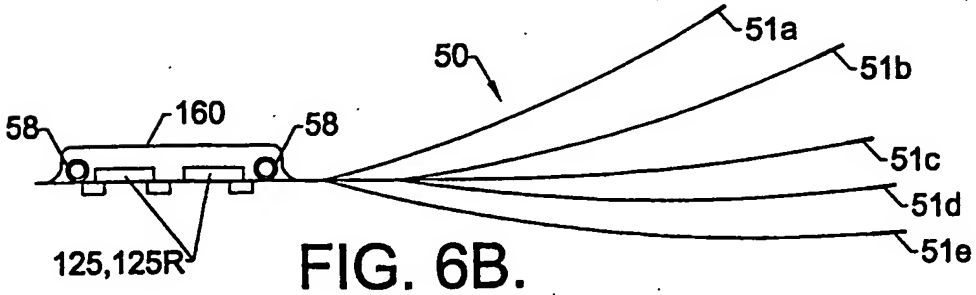


FIG. 6B.

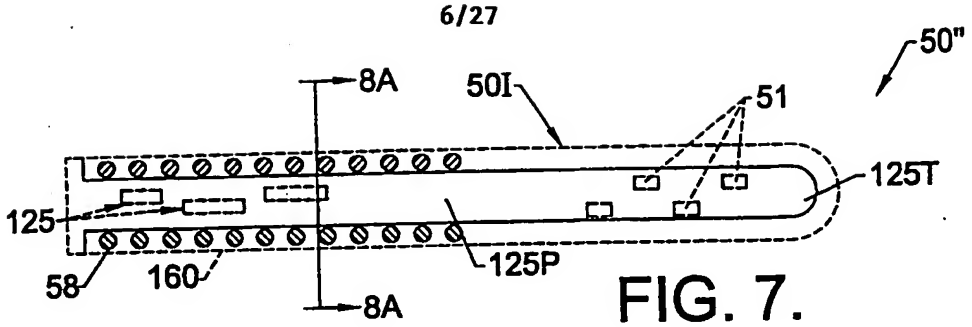


FIG. 7.

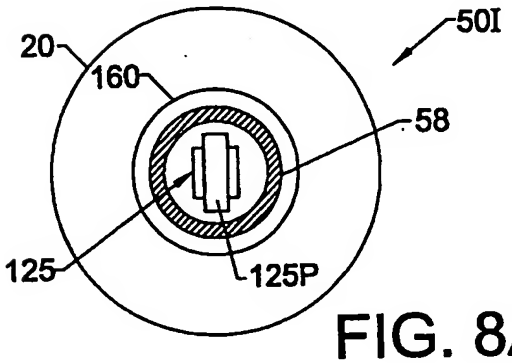


FIG. 8A.

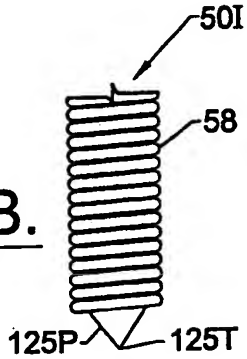


FIG. 8B.

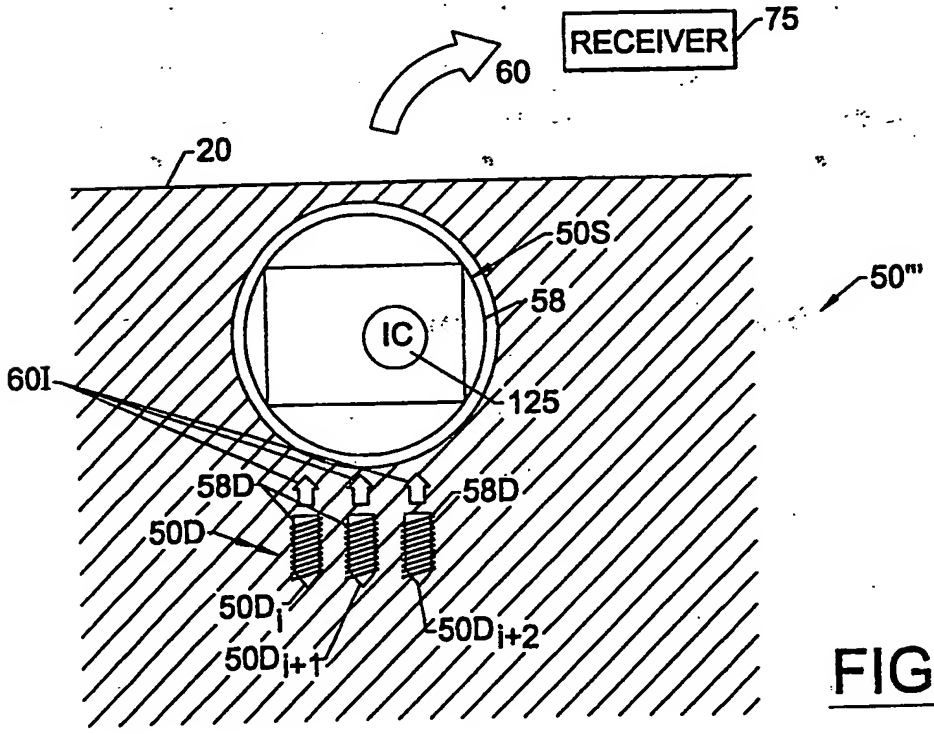


FIG. 9.

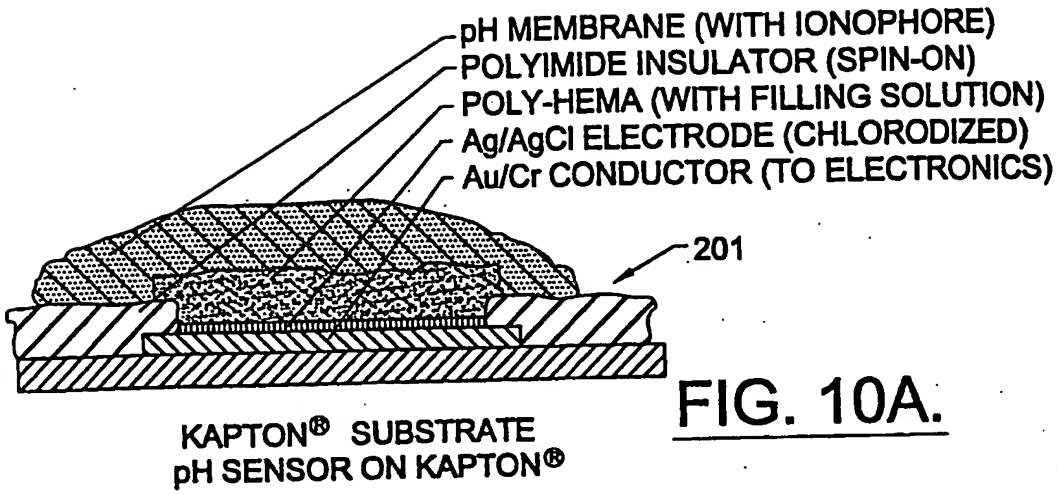


FIG. 10A.

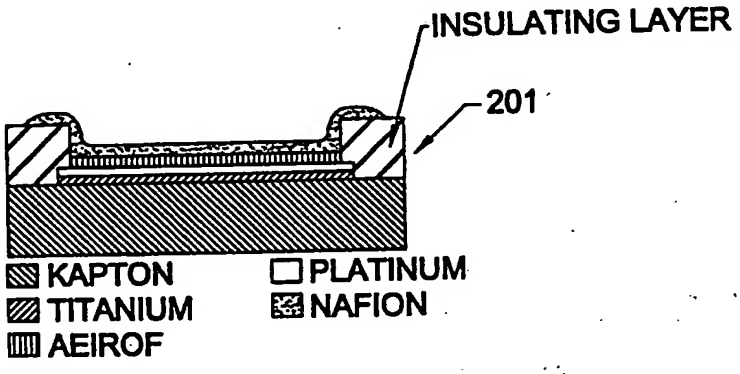


FIG. 10B.

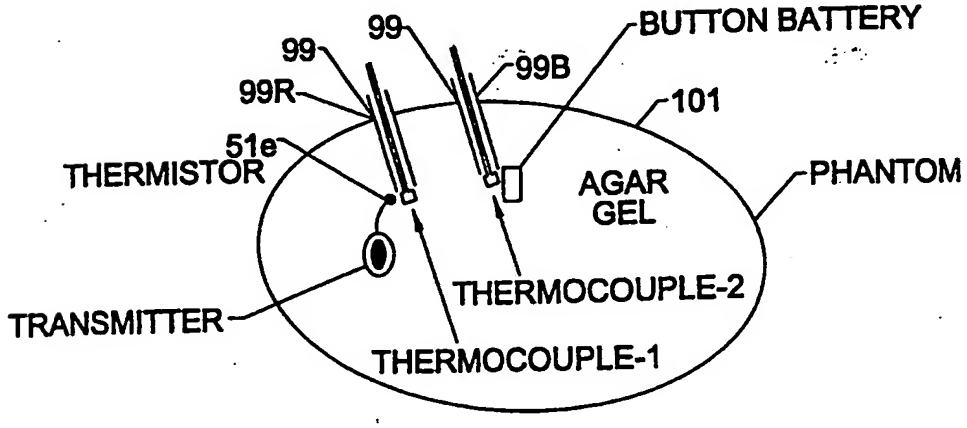


FIG. 11.

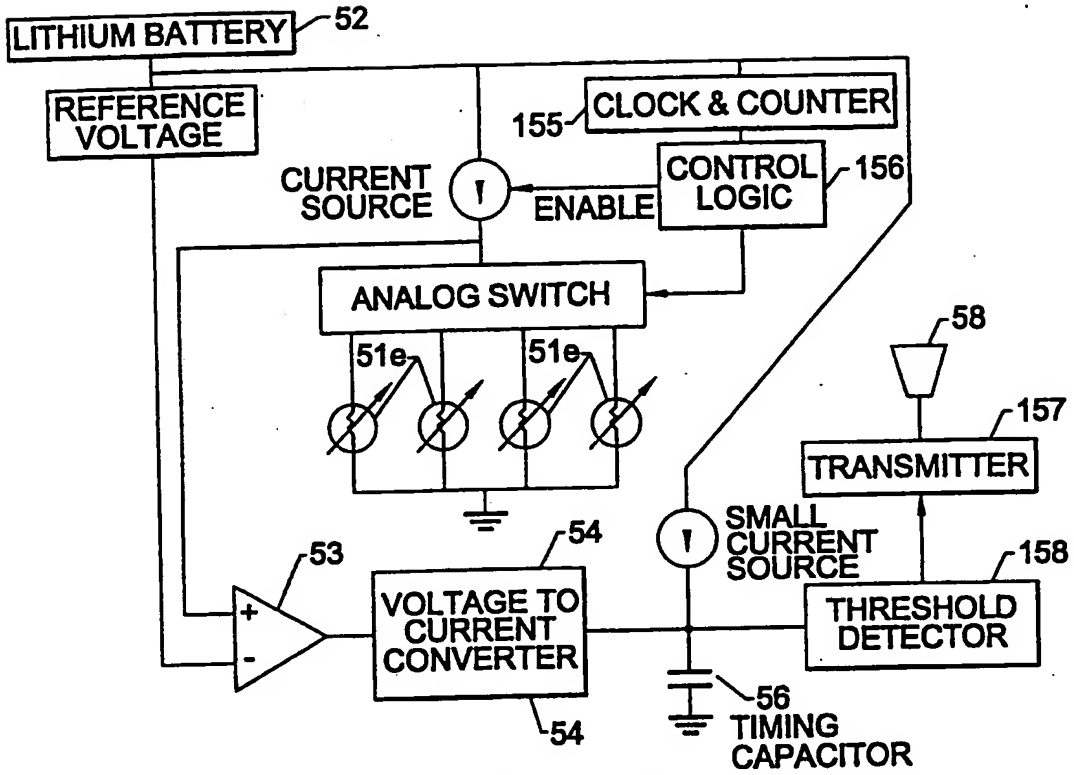

LITHIUM BATTERY
52
REFERENCE VOLTAGE
155
CLOCK & COUNTER
CURRENT SOURCE
ENABLE
CONTROL LOGIC
156
ANALOG SWITCH
51e
51e
58
157
TRANSMITTER
SMALL CURRENT SOURCE
158
53
54
VOLTAGE TO CURRENT CONVERTER
THRESHOLD DETECTOR
56
TIMING CAPACITOR
54

FIG. 12.

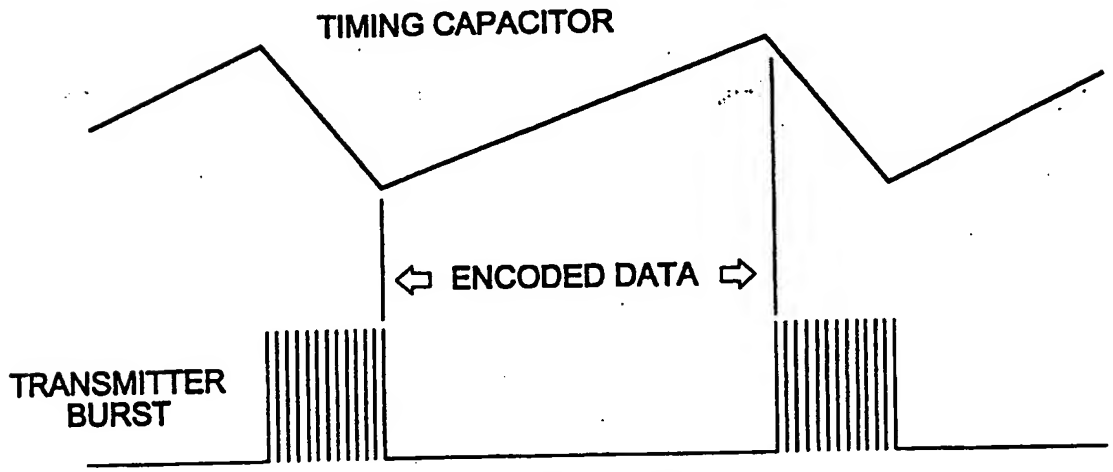

TIMING CAPACITOR
ENCODED DATA
TRANSMITTER BURST

FIG. 13.

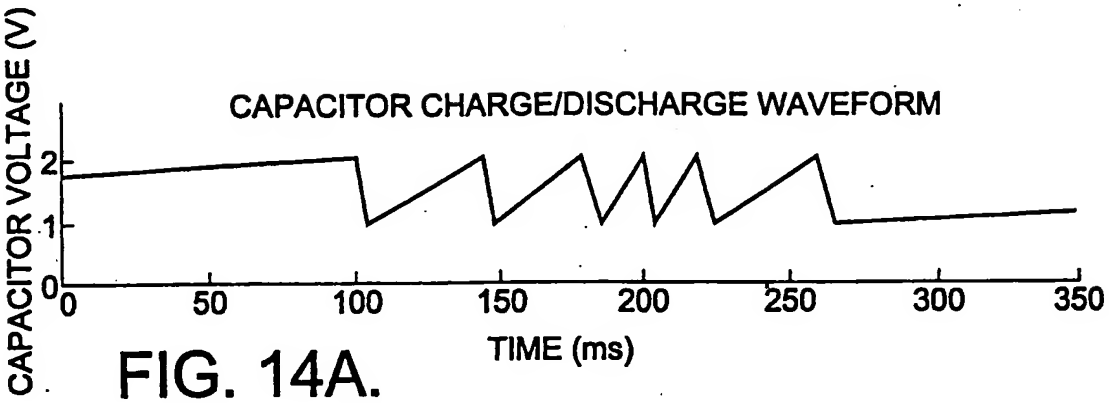


FIG. 14A.

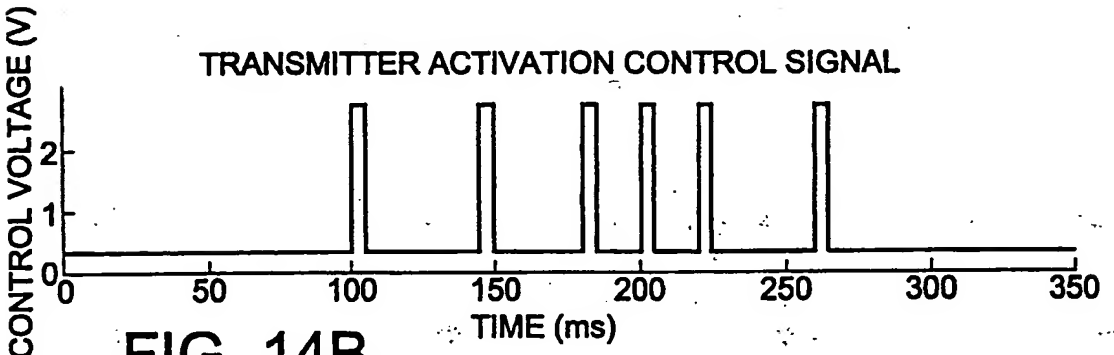


FIG. 14B.

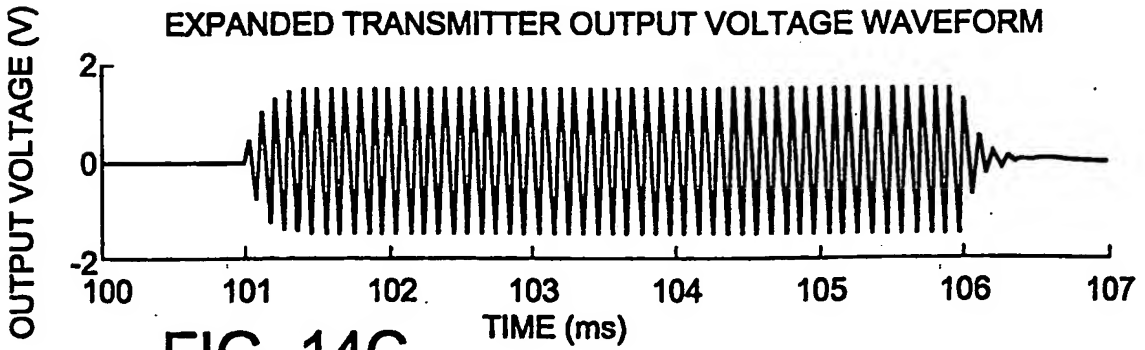
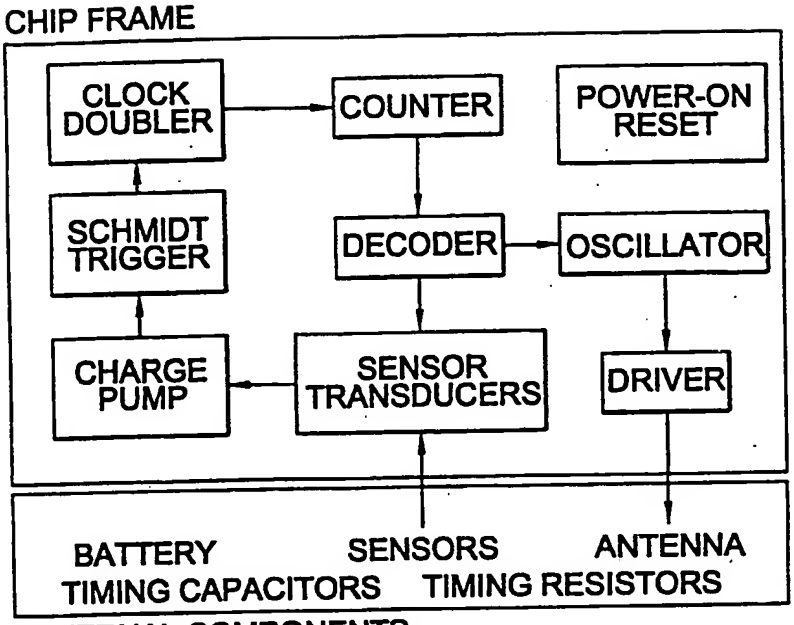
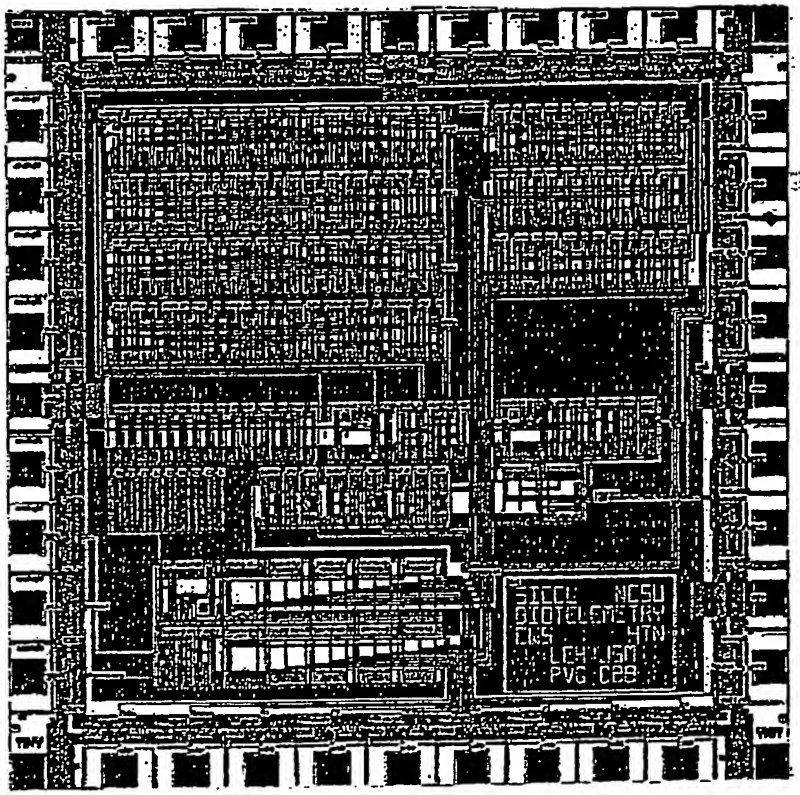


FIG. 14C.



IC BLOCK DIAGRAM

FIG. 15.



IC LAYOUT

FIG. 16.

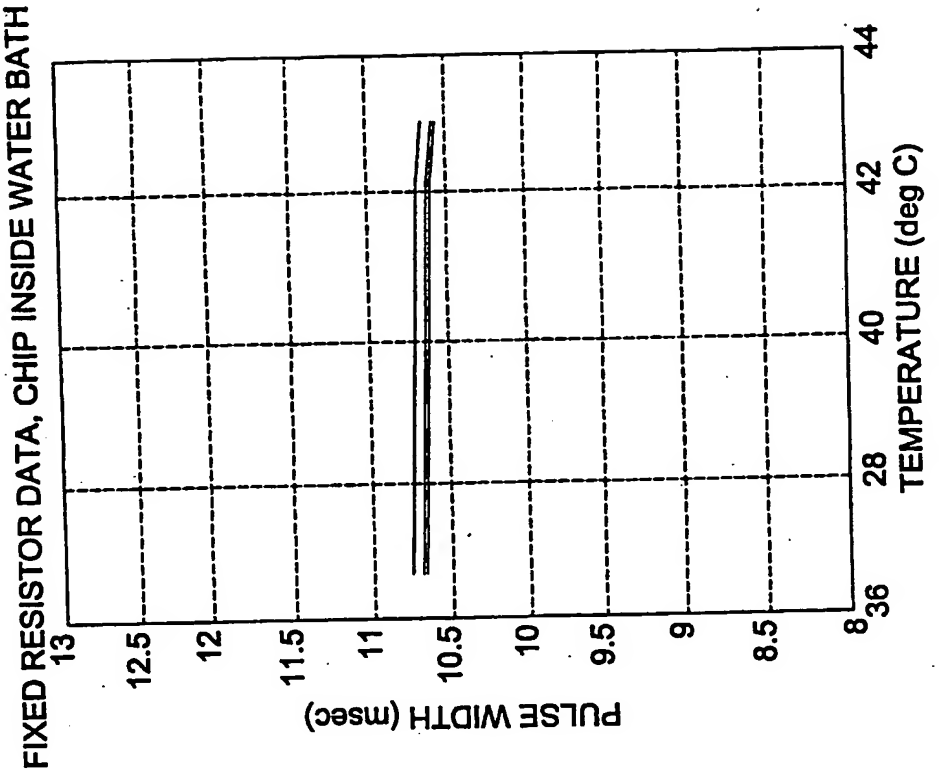


FIG. 17B.

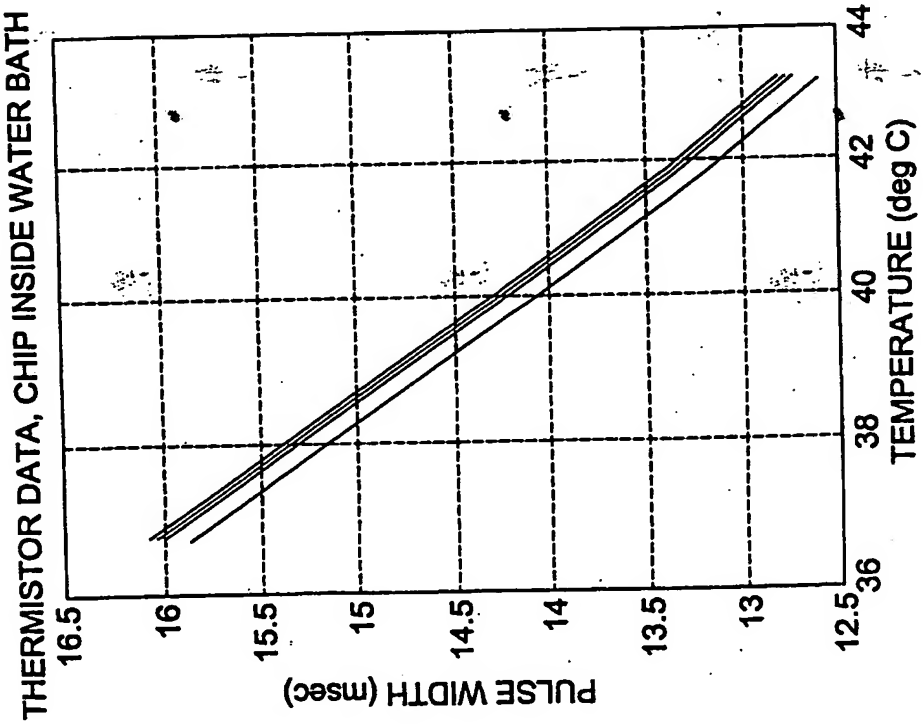


FIG. 17A.

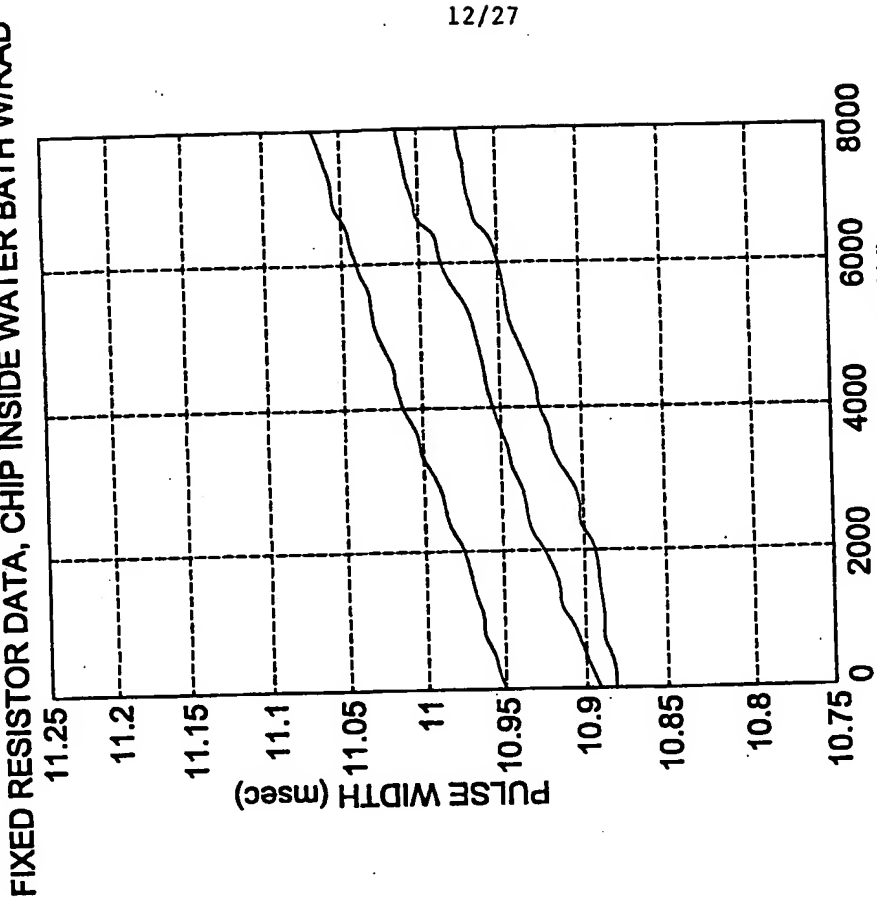


FIG. 18B.

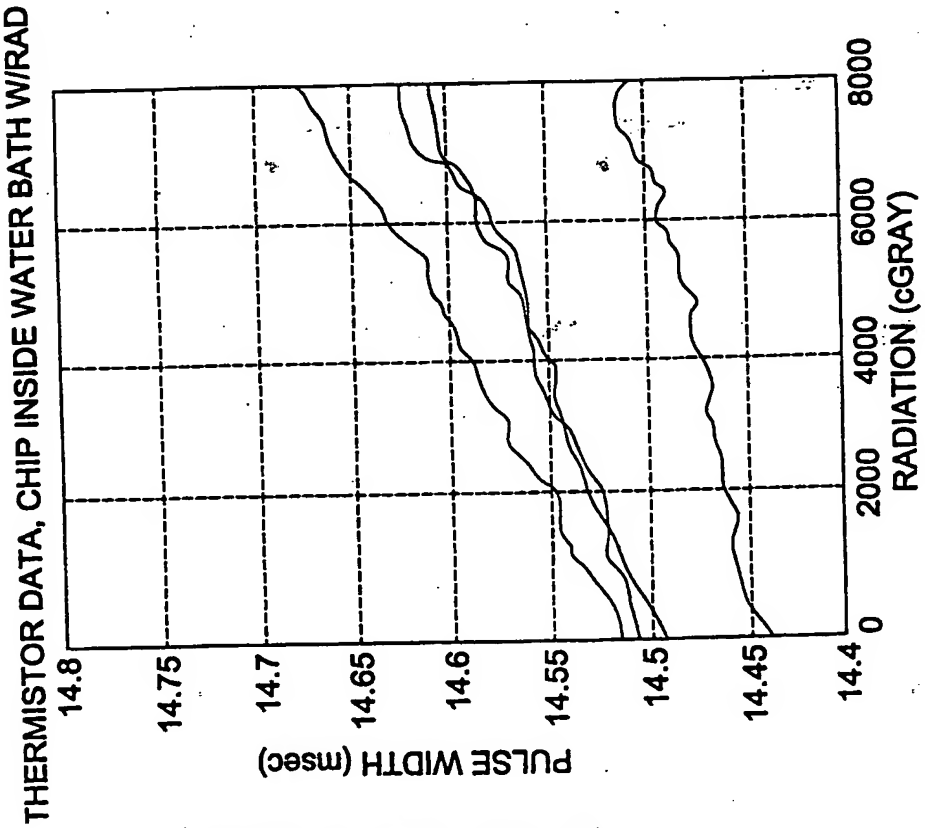


FIG. 18A.

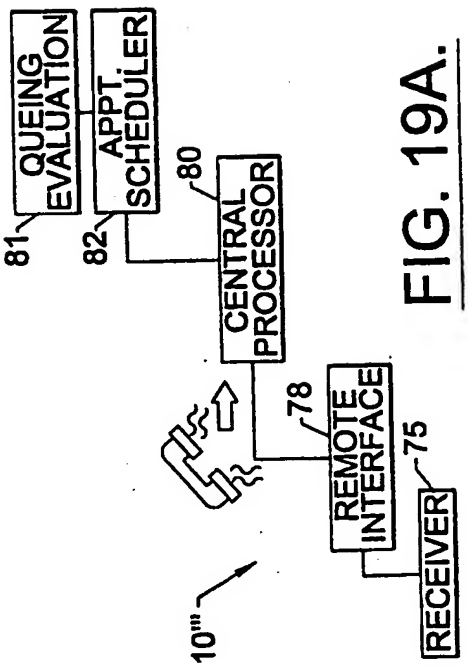


FIG. 19A.

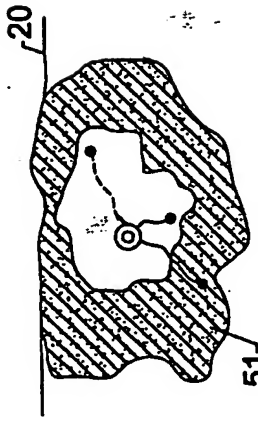
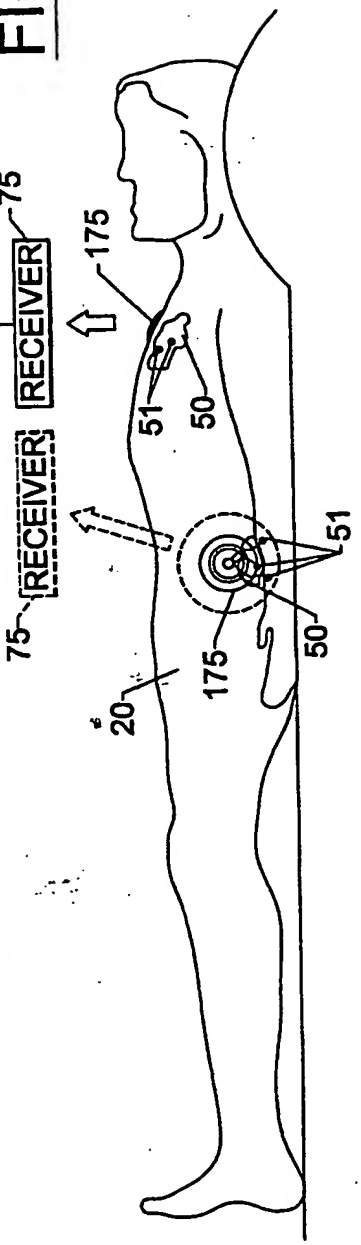
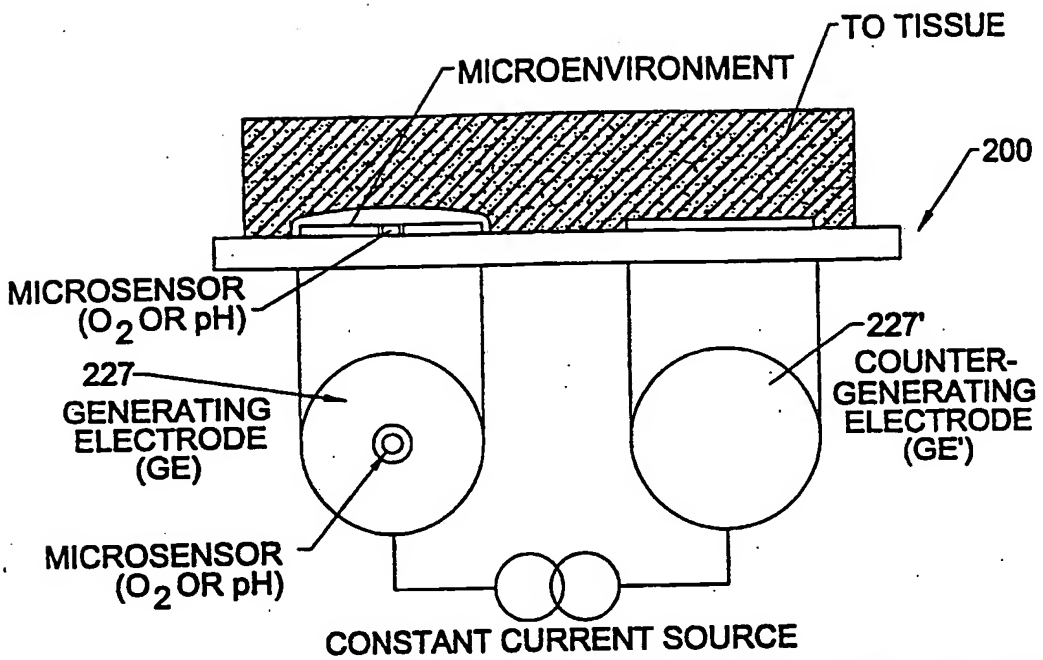


FIG. 19B.





OPERATING PRINCIPLE OF SELF-CALIBRATING, *IN SITU, IN VIVO* MICROSENSOR. THE MICROENVIRONMENT CONTROLLED BY THE MAGNITUDE AND DIRECTION OF THE GENERATING CURRENT; OXYGEN-SATURATION OR OXYGEN-DEPLETION DEPENDS ON ANODIC OR CATHODIC BEHAVIOR OF GE.

FIG. 20.

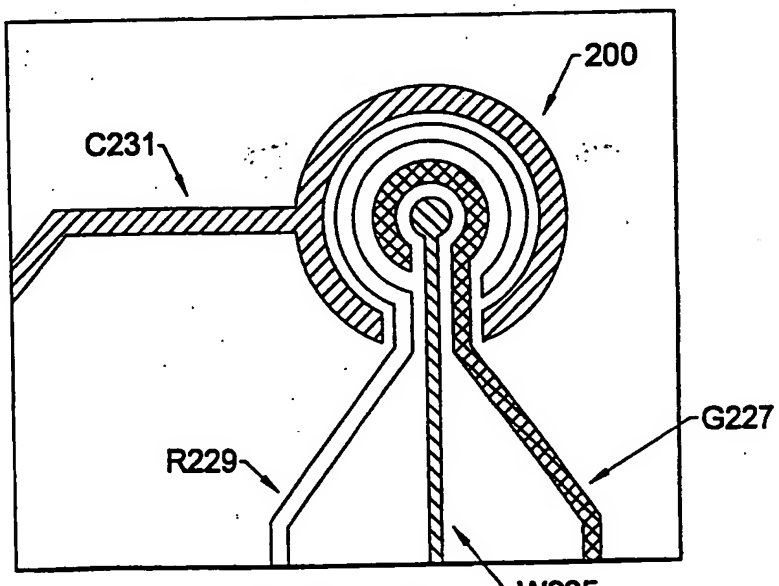


FIG. 21.

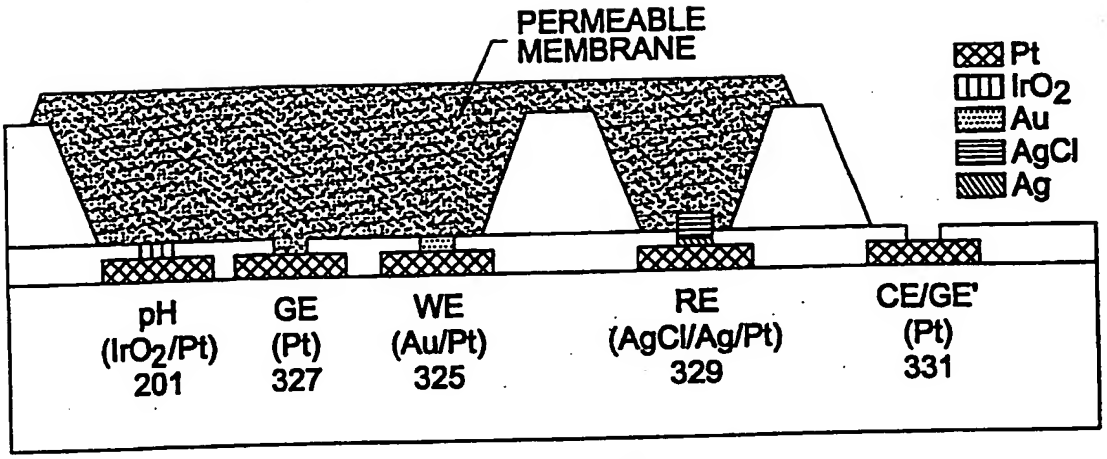
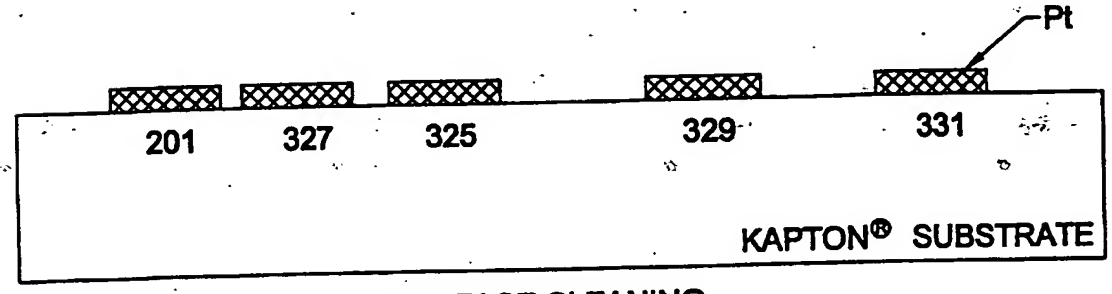
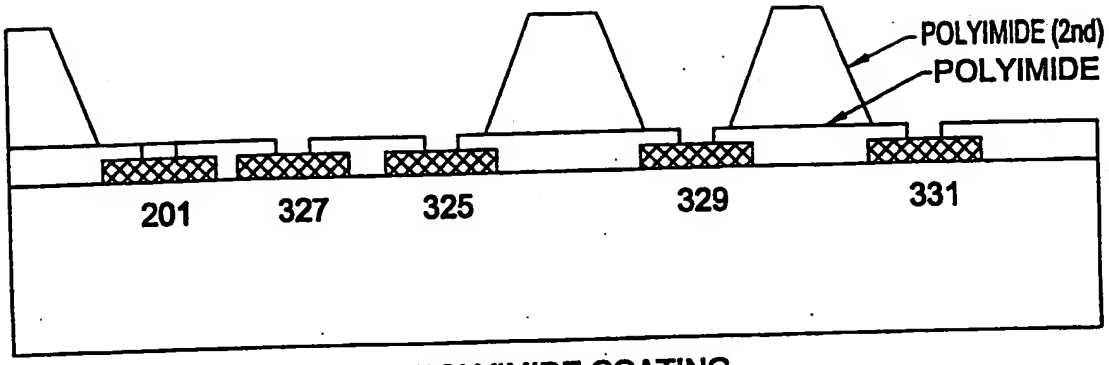


FIG. 22.



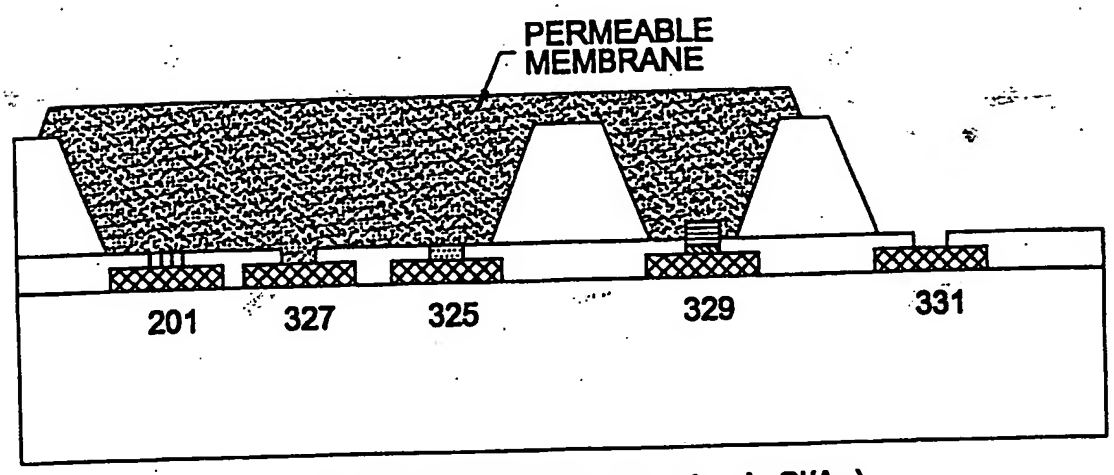
SURFACE CLEANING
METAL (Pt) DEPOSITION
PHOTOLITHOGRAPHY
METAL ETCHING

FIG. 23A.



POLYIMIDE COATING
POLYIMIDE LITHOGRAPHY
POLYIMIDE COATING (2nd)
POLYIMIDE LITHOGRAPHY

FIG. 23B.



ELECTROPLATING (InO_2 ,Au, AgCl/Ag)
PERMEABLE MEMBRANE CASTING

FIG. 23C.

FIG. 24

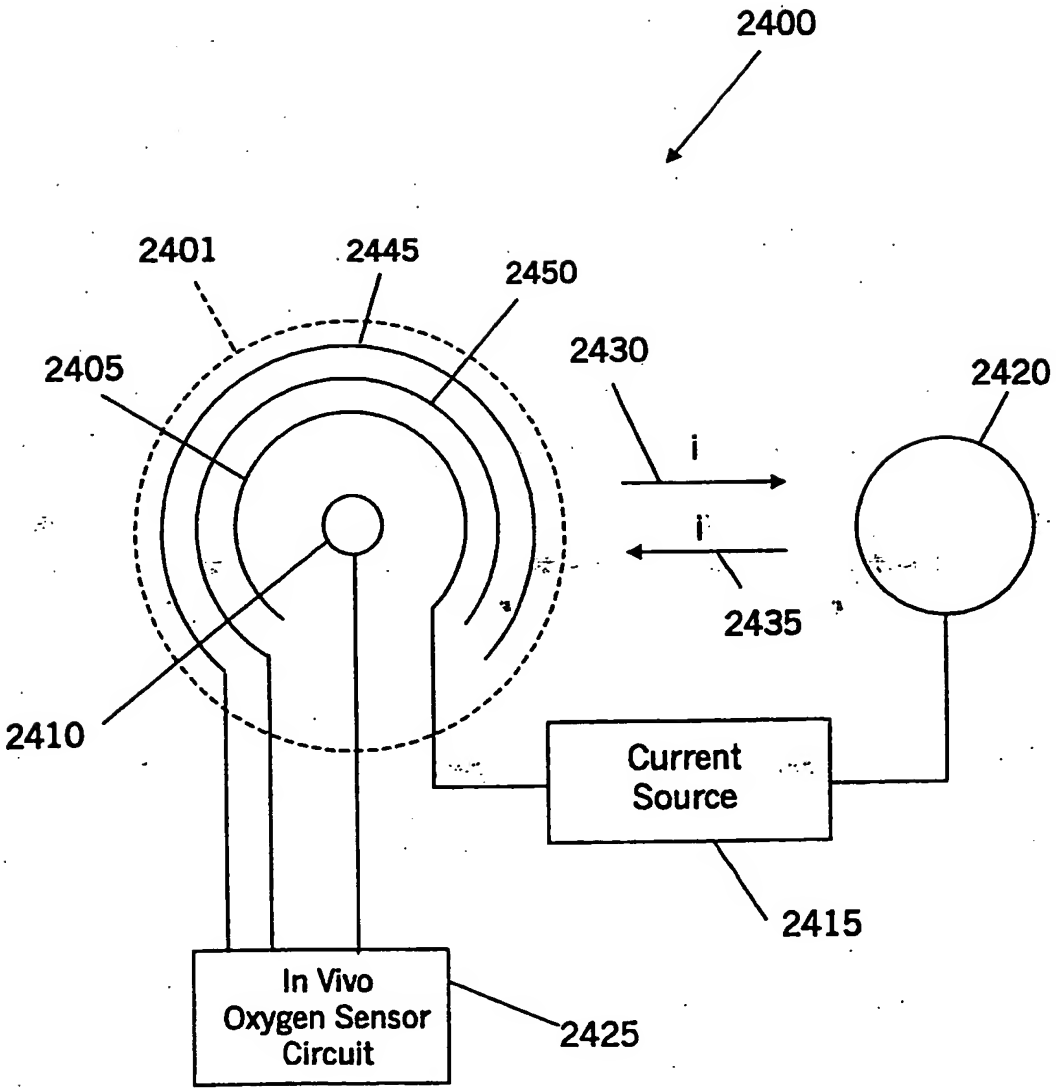

2400
2401
2445
2450
2405
2430
2420
i
i
2435
2410
Current Source
2415
In Vivo Oxygen Sensor Circuit
2425

FIG. 25A

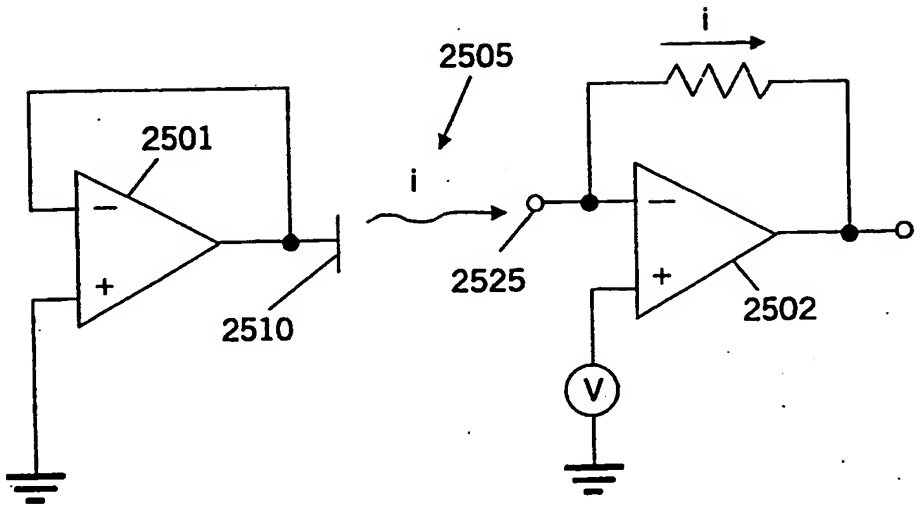


FIG. 25B

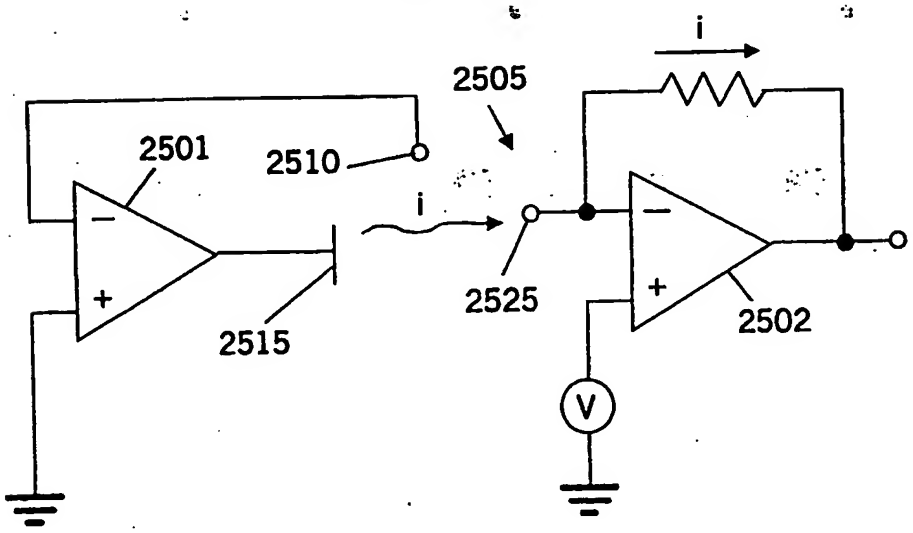


FIG. 26

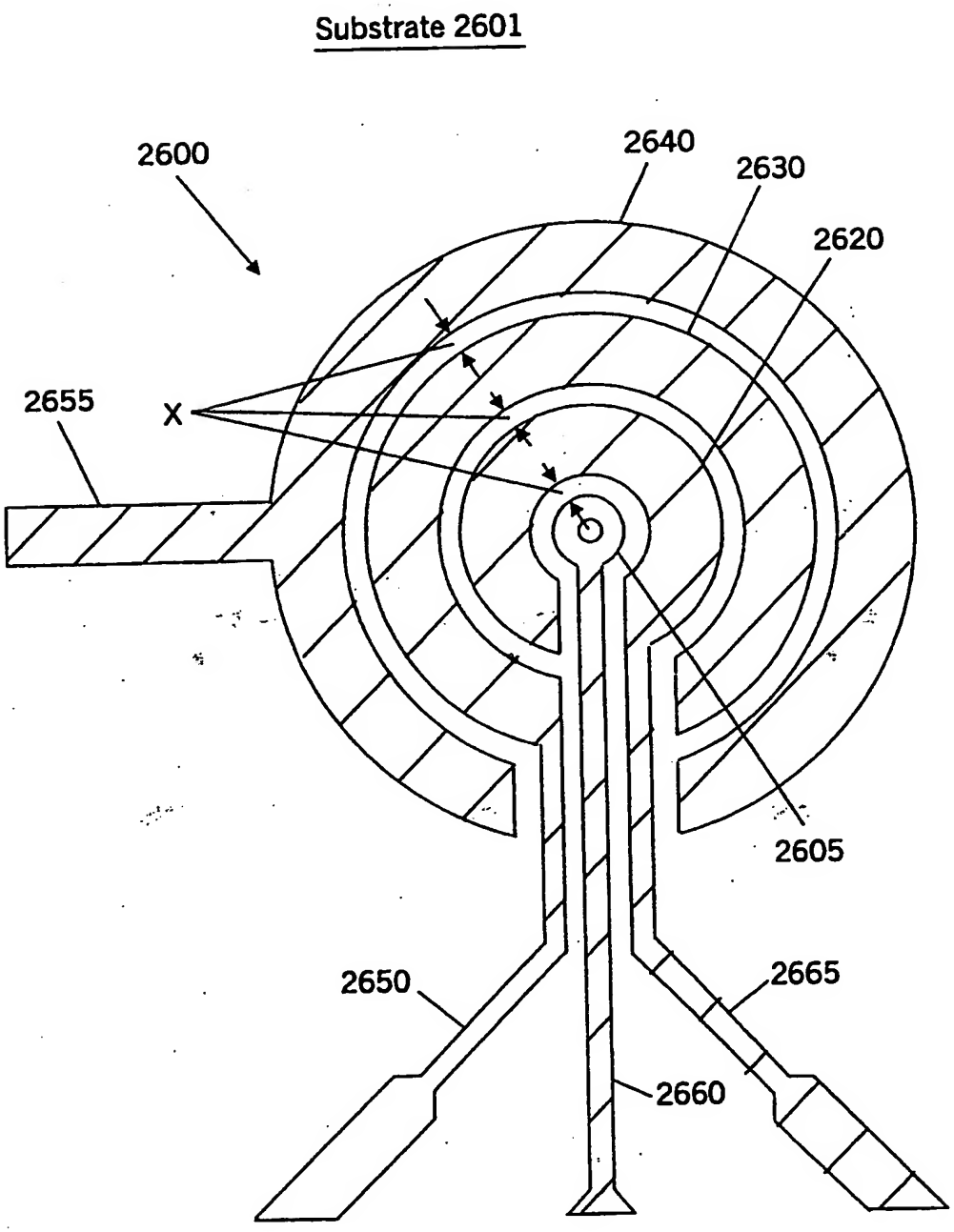

Substrate 2601
2600
2640
2630
2620
2655
X
2605
2650
2665
2660

FIG. 27

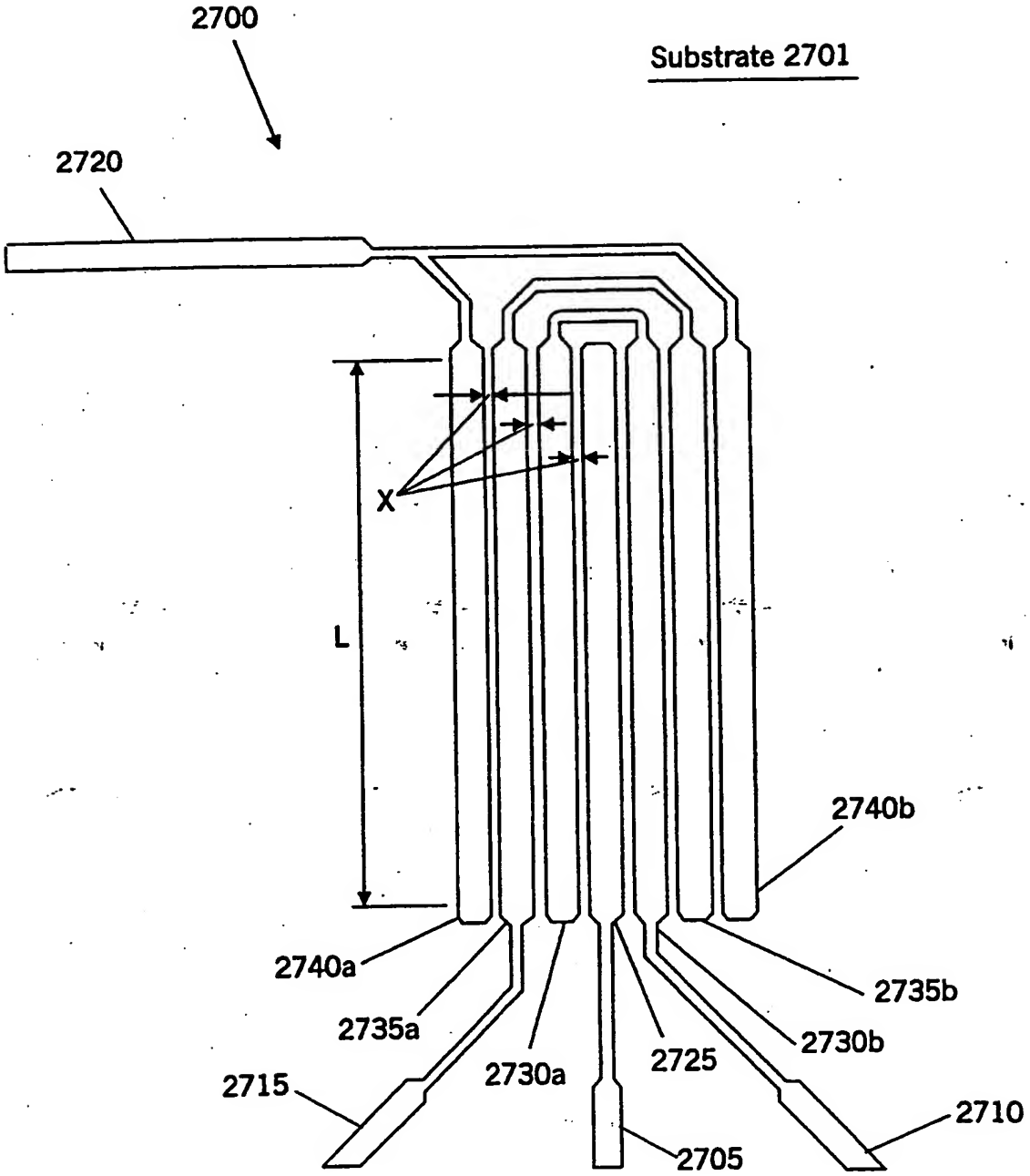

2700
Substrate 2701
2720
X
L
2740b
2740a
2735b
2735a
2730b
2715
2730a
2725
2710
2705

FIG. 28

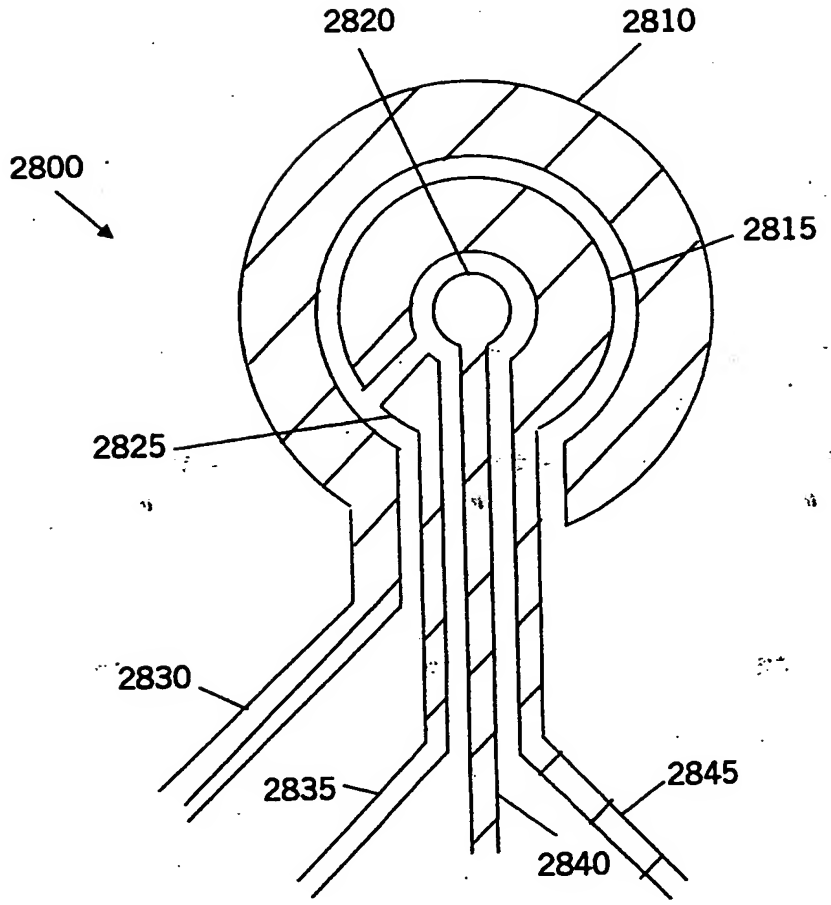


FIG. 29

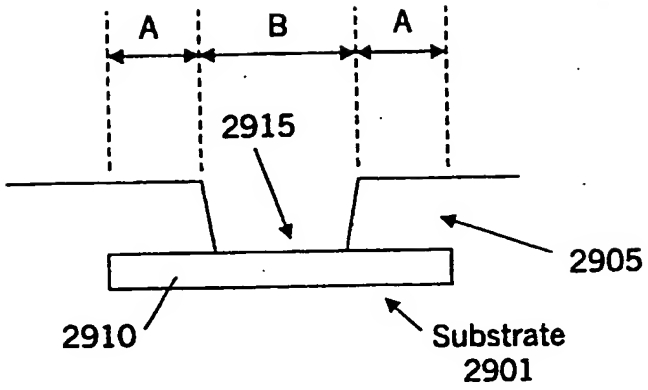

A
B
A
2915
2905
2910
Substrate
2901

FIG. 31

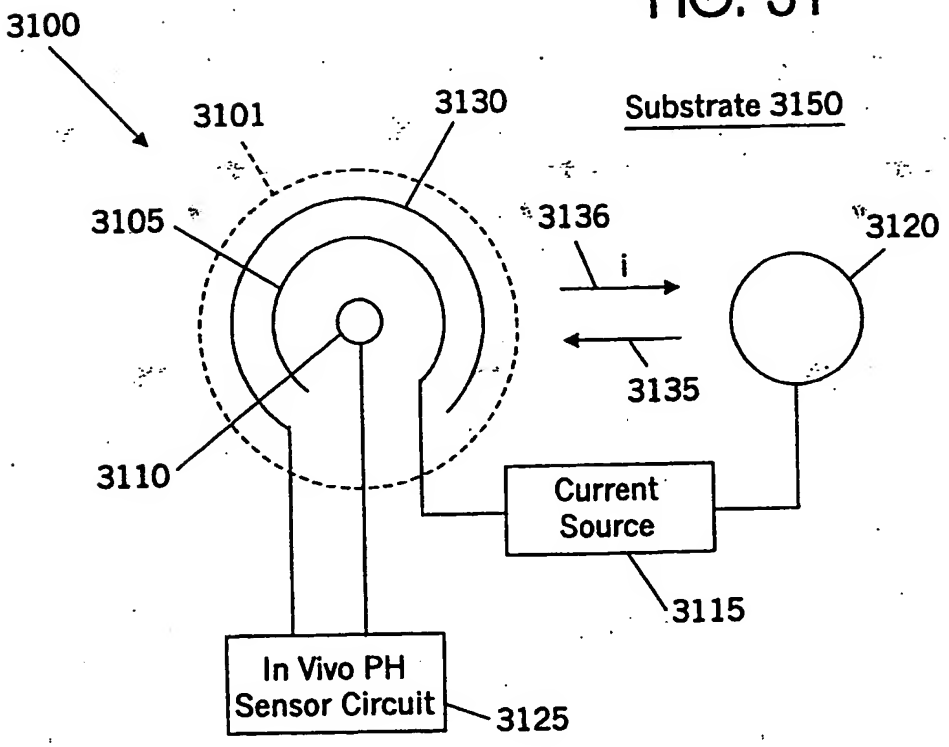

3100
3101
3130
Substrate 3150
3105
3136
3120
i
3135
3110
Current
Source
3115
In Vivo PH
Sensor Circuit
3125

FIG. 30

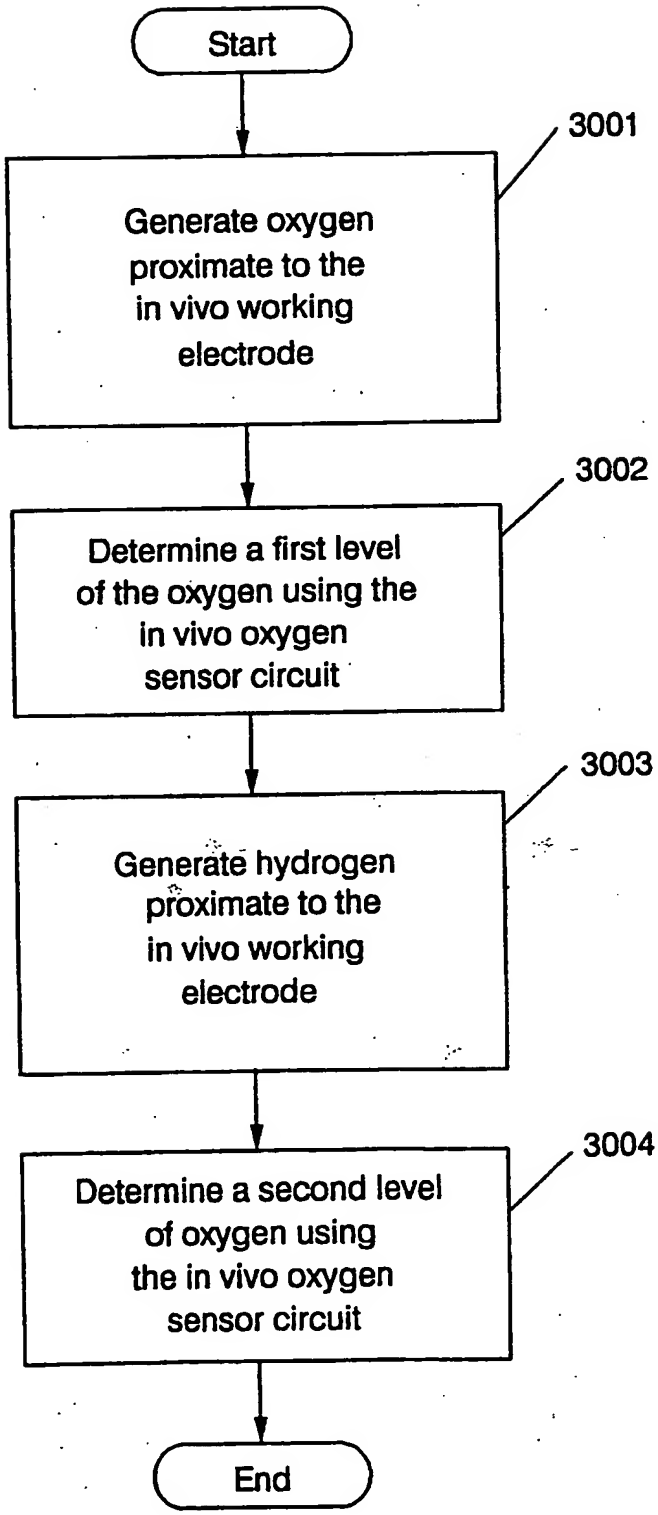

Start
3001
Generate oxygen proximate to the in vivo working electrode
3002
Determine a first level of the oxygen using the in vivo oxygen sensor circuit
3003
Generate hydrogen proximate to the in vivo working electrode
3004
Determine a second level of oxygen using the in vivo oxygen sensor circuit
End

FIG. 32

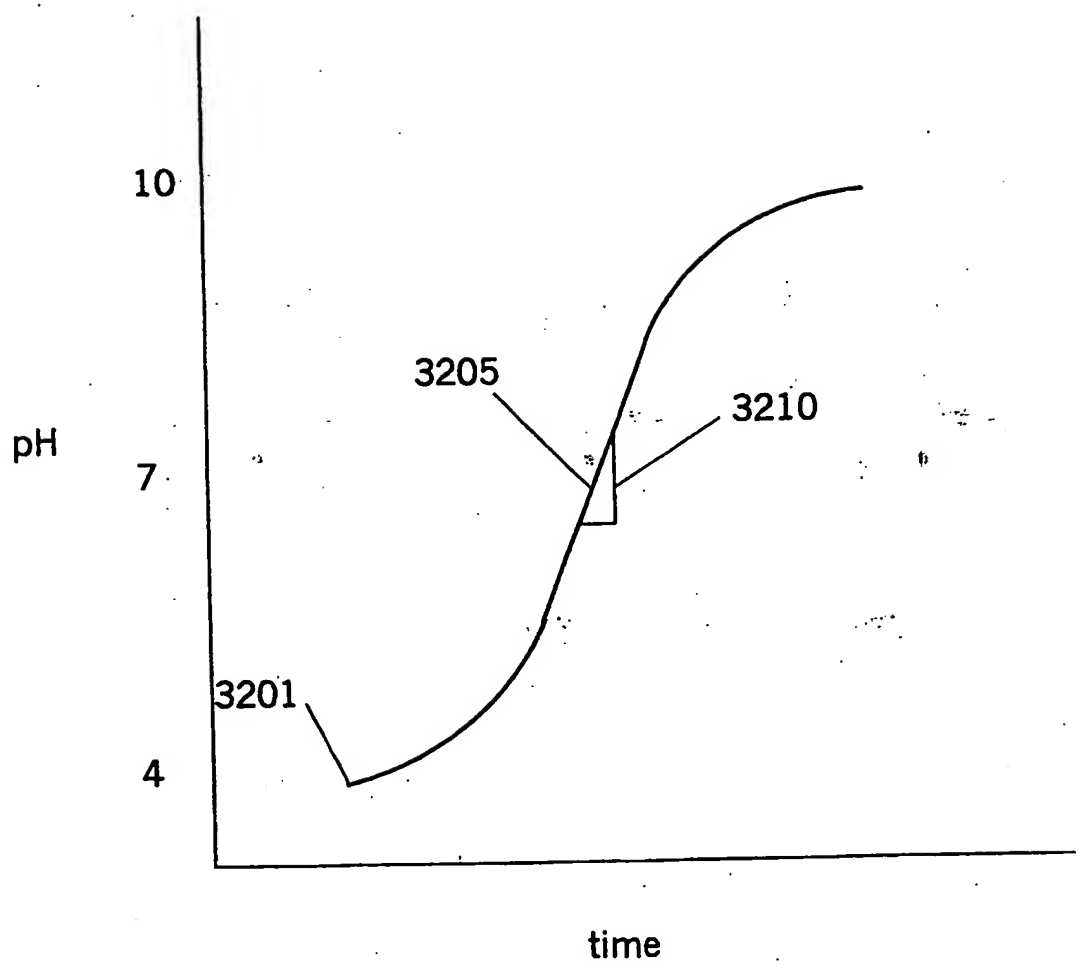


FIG. 33

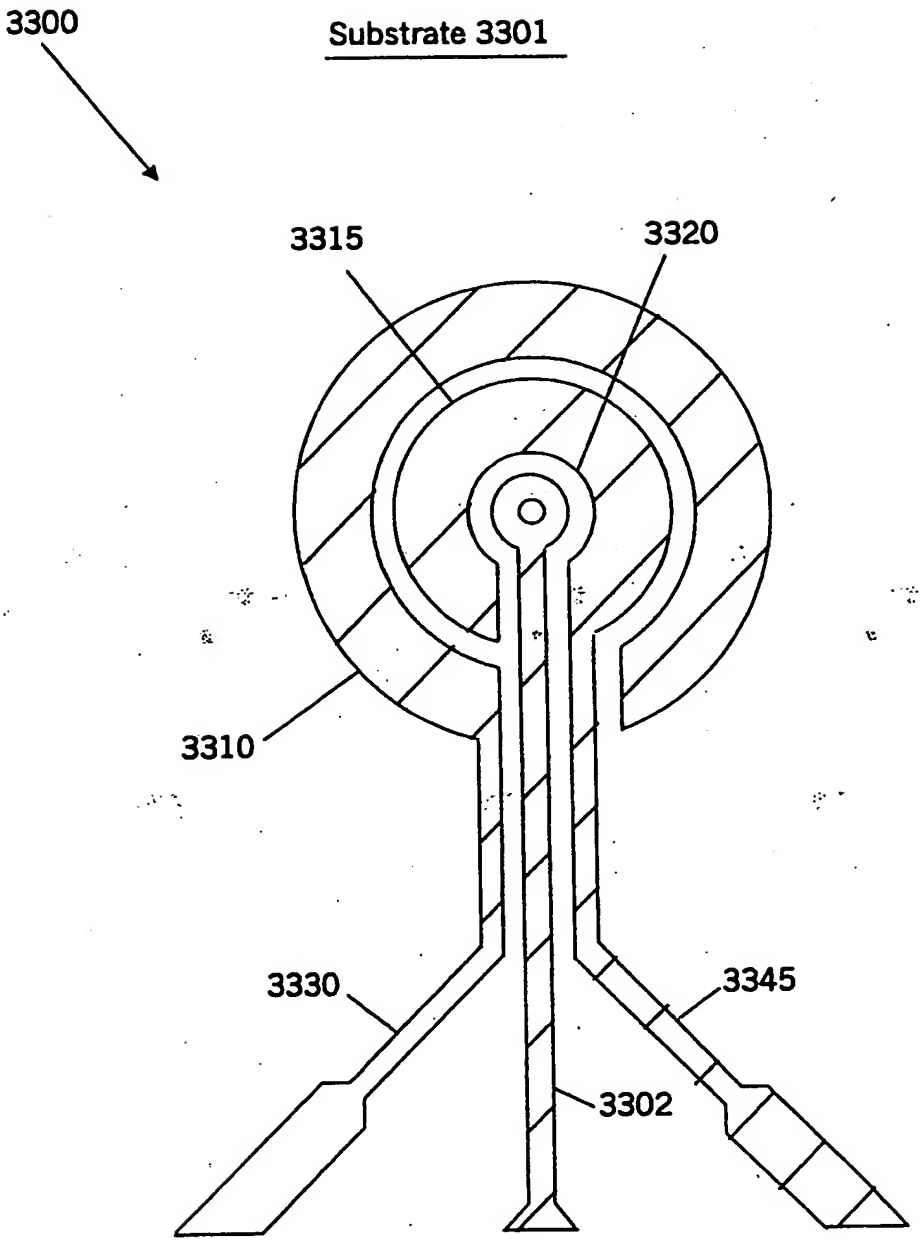


FIG. 34

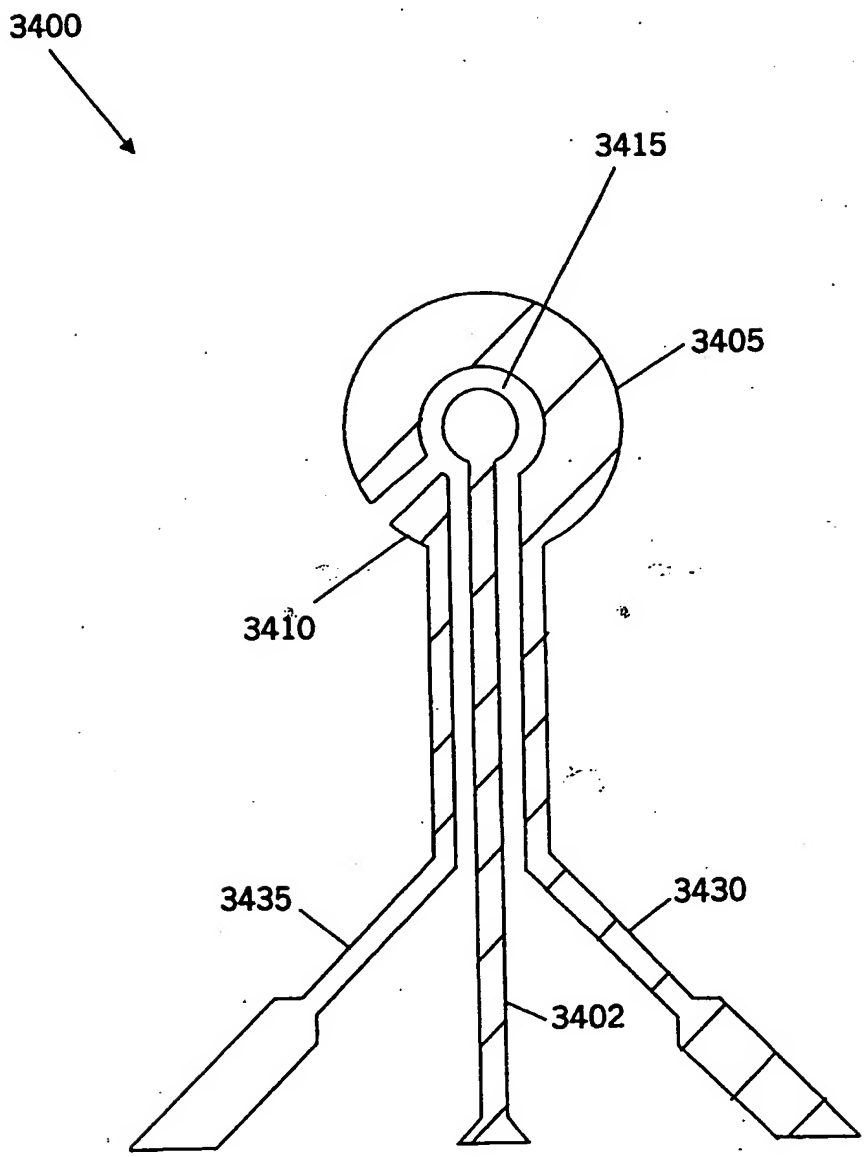
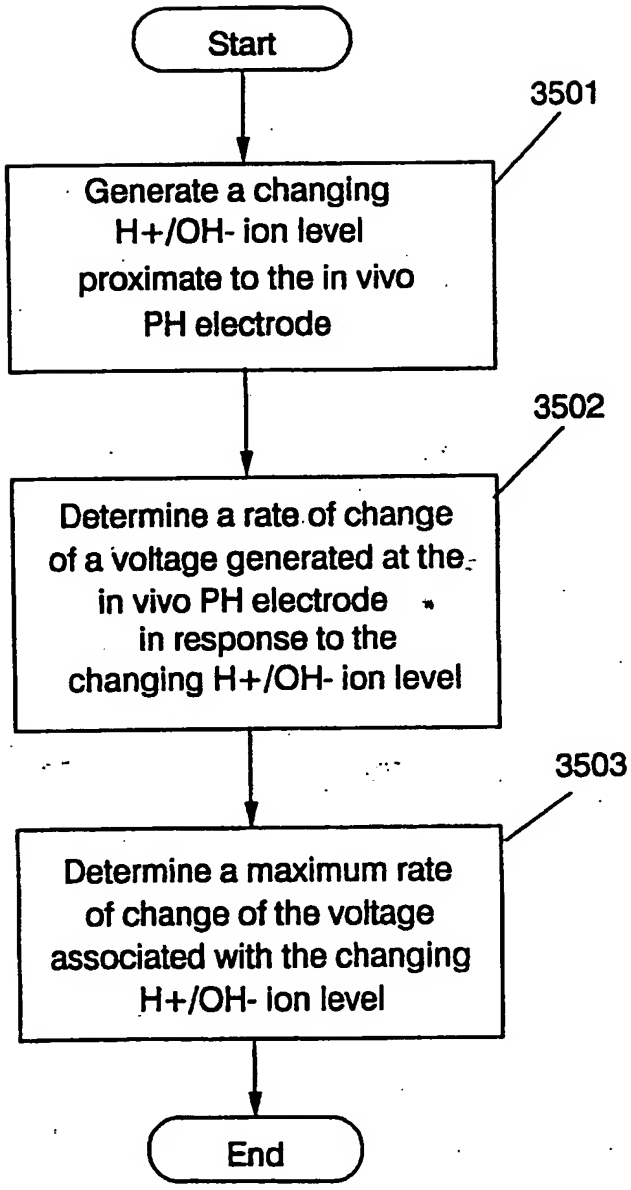


FIG. 35


Start
3501
Generate a changing H+/OH- ion level proximate to the in vivo PH electrode
3502
Determine a rate of change of a voltage generated at the in vivo PH electrode in response to the changing H+/OH- ion level
3503
Determine a maximum rate of change of the voltage associated with the changing H+/OH- ion level
End

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 00/08310

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61B5/00 C12Q1/00 G01N27/52

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61B C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	WO 98 58250 A (ELAN CORP.) 23 December 1998 (1998-12-23) page 3, line 25 -page 16, line 8 page 18, line 7 -page 30, line 20	1-5,7, 10,12 6,11, 13-21, 24-31
X	US 4 655 880 A (CASE WESTERN) 7 April 1987 (1987-04-07) column 4, line 2 -column 6, line 11 column 13, line 19 -column 14, line 58	32,41
X A	US 4 571 292 A (CASE WESTERN) 18 February 1986 (1986-02-18) column 10, line 23 - line 37	32 33-41

-/—

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 October 2000	25. 10. 2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Lemercier, D

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INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 00/08310

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 537 761 A (MATSUSHITA) 21 April 1993 (1993-04-21) column 6, line 14 -column 18, line 58	32-41

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INTERNATIONAL SEARCH REPORT

Int. ational application No.
PCT/US 00/08310

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

As a result of the prior review under R. 40.2(e) PCT, no additional fees are to be refunded.

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☒ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-31

   Methods of calibrating an in vivo sensor system

2. Claims: 32-41

   Electrode assembly

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte onal Application No
PCT/US 00/08310

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		AU 8031898 A	04-01-1999
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EP 537761 A	21-04-1993	DE 69221808 D	02-10-1997
		DE 69221808 T	02-04-1998
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